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Chemotherapeutic Activity of 1,4-Dichloro-2,4-Dimethoxybenzene (Demosan) and Other Compounds Against Rhizoctonia Solani in Cotton Seedlings.

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ABSTRACT

The development of a glass dish and a flask technique for use in the laboratory, and a clay pot technique for use in the greenhouse proved to be satisfactory for the study of systemic chemotherapeutic activity of Demosan, Vitavax and Plantvax against Rhizoctonia solani on cotton seedlings.

Laboratory experiments showed that when used as seed treatments, Demosan protected cotton seedlings for about 10 days, Vitavax for about 3 weeks, and Plantvax for about 2 weeks.

Greenhouse experiments showed, when they were used as seed treatments, Demosan protected cotton seedlings for about 2 weeks, Vitavax for about 3 weeks, and Plantvax for about 2 weeks. When they were used as soil treatment, Demosan appeared to stimulate seed germination at lower rates and gave protection for about 2 weeks at 0.64 gm/pot of formulated material. Phytotoxicity, however, by Demosan was noted when a higher rate (0.96 gm/pot) was used. Vitavax gave protection for over 4 weeks, Plantvax gave protection for over 3 weeks when either was added to soil at a rate of 0.15 gm/pot. Delayed germination by both fungicides was observed.

Results from studies: (1) using the flask technique; (2) concerned with the treated-tissue-extract experiments; and (3) using histological techniques, showed when cotton seedlings were exposed to Demosan that the hypocotyl tissue was protected against infection by R. solani. This indicated absorption by the roots and translocation into the hypocotyl of a systemic fungicide.

Tissue from seedlings exposed to Demosan showed a significant reduction in the amount of reducing sugars 15 days after treatment. There was no significant reduction at 7 days.

The fungicide was considered to be fungistatic based on in vitro studies.

INTRODUCTION

The application of protectant fungicides to plants, seed, and soil has offered effective control for a number of plant pathogens. This method of disease control has a number of serious limitations. Coverage of the exposed plant surface above the ground is seldom complete, leaving certain areas, as well as the new growth unprotected. Protective fungicides by definition do not eradicate established infections. The search for chemotherapeutants which would not have these limitations has given the impetus for the successful development of systemic pesticides.

A succession of pesticidal compounds has evolved in the past 25 years each with unique qualities when compared with preceding types. Within the past three years a number of "systemic fungicides" have been developed by industrial firms. Their unique properties offer a great future for the development of more efficient methods for controlling plant diseases. Sharvelle (114) stated in 1961, that: "The use of fungicides for systemic protection in which the chemical is introduced or absorbed into the plant system, acting as 'vaccines' against plant diseases, is still a relatively new and underdeveloped possibility." This is no longer the situation.

The study of systemic fungicides comes under a general heading of chemotherapy for control of plant diseases. Horsfall and Dimond (71, 72) defined chemotherapy as the control of plant disease by compounds that, through their effect upon the host or pathogen, reduce or nullify the effects of the pathogen after it has entered the plant. The compound that initiates this effect, either directly,

or indirectly, is called a chemotherapeutant. Horsfall (70) distinguished two different types of chemotherapy: (a) "Topical chemotherapy" in which the chemotherapeutant penetrates only a small distance into the plant and has a local effect, and (b) "Systemic chemotherapy" in which the chemical is translocated to the various parts of the plant.

There is discrepancy in the literature with respect to the terms: "chemotherapeutant" and "systemic fungicide." In some instances these terms have been used interchangeably. Cremlyn (15) defined a systemic fungicide as a compound which is taken up by the plant and translocated within the plant system and either protecting it from attack by pathogenic fungi, or limiting an already established infection. An examination of this definition indicates a close agreement with the definition of systemic chemotherapeutant suggested by Horsfall (70). The term "systemic fungicide" however, gives the connotation that the compound is translocated in the plant and only acts directly on the pathogen. The compound, however, may act either or both as a protectant and an eradicant in the host tissue.

Three mechanisms were proposed to account for plant chemotherapy: (a) the compounds act directly on the pathogen in the host; (b) they neutralize a toxin produced by the pathogen; or (c) they act on host plant to increase resistance to disease (28, 31, 70, 71, 72).

There appears to be three main reasons for the slow evaluation of the development of systemic compounds for control of plant diseases (27). First, various plant pathogens are different in character which may be responsible for the selective toxicity of certain compounds.

Second, the requirements for a systemic compound are much more rigorous than for a protective or an eradicative fungicide. Third, the systemic compound must be translocatable, and most important, it must be toxic only to the pathogen and not the host at the concentrations used.

Systemic control of plant diseases is appealing to plant pathologists because theoretically it provides means to control certain plant pathogens that invite such diseases as vascular wilts, root rots, and diseases caused by obligate parasites, which, until now have been difficult or impossible to control. Recently a number of compounds have become available for experimental and commercial use which exhibit systemic activity. Finally, it appears that the systemic compounds now in use, once applied to the plant, are readily metabolized by the host and are therefore, less hazardous to man and other animals than the conventional protectants and eradicants. It is interesting to note that some of the most active systemic compounds used in the control of plant diseases are "antibiotics." Antibiotics have probed to be too expensive for commercial use.

Cotton Seedling Diseases and Their Economic Importance

Rhizoctonia solani causes damping-off and soreshin diseases of cotton seedlings. It is one of the most important if not the most important pathogen in the cotton seedling disease complex. The term "soreshin" has been in use since 1892, when Atkinson (7) isolated a fungus from diseased cotton seedlings and proved that it was responsible for the damping-off or soreshin of cotton. Edgerton (100), in 1911, reported the Rhizoctonia disease of cotton in Louisiana.

It is interesting to note in the literature that Rhizoctonia solani was not always considered as an important cotton seedling pathogen. Haskell and Wood (67), in 1927, reported considerable damage of cotton from soreshin disease in the Southern cotton growing states. Arndt (5), in 1935, found R. solani damage on less than 10 per cent of the diseased hypocotyls of cotton seedlings he examined during the period 1924 to 1934. Lehman (85), in 1938, and Miller and Weindling (93), in 1940, reported very little damage of cotton seedlings by R. solani. However by 1942, Ray and McLaughlin (105), reported that R. solani was the second most commonly isolated fungus from diseased cotton seedlings in Oklahoma. More recently, Leyendecker (87), Ranny (104) and Smith (129) indicated that the seedling disease complex, including R. solani caused the greatest loss of cotton in terms of yield. According to Arndt (6), R. solani is generally considered to be the cause of greater losses of cotton seedlings than any other pathogen when likelihood of infection by the anthracnose fungus (Glomerella gossypii) is eliminated by seed treatment. R. solani was one of the most frequently isolated pathogens from the diseased cotton seedlings in Fulton and Bollenbachen's (51), and Sinclair's (120) studies. Within the last decade, with the cost of production increasing and the profits decreasing, the economic importance of soreshin and other seedling diseases of cotton has gained recognition (120). Losses due to cotton seedling diseases are of great importance in Louisiana and account for approximately 5.0-6.0 percent of the total cotton yield reduction caused by diseases (10, 120).

Cotton seedling diseases are caused by several nonspecialized soil-borne fungi, and is referred to as the "seedling disease complex." The cotton seedling disease cycle begins at planting and continues throughout the seedling stage. Seedling injury takes several forms, each characterized by distinctive symptoms such as: seed rot, pre-emergence damping-off, seedling root rot, and post-emergence damping-off. Symptoms of post-emergence damping-off may appear at any time during the first part of the growing season. A number of pathogens were found to be associated with this phase of the disease complex, including species of Rhizoctonia, Pythium, Colletotrichum, Aspergillus and Penicillium. Isolations from diseased cotton seedlings collected from Louisiana fields showed that there were at least six fungi involved (120). Rhizoctonia solani was found to be a major con-contributor to the cause of these diseases, especially post-emergence damping-off. Most of the post-emergence damping-off occurs in the cotton seedlings before the formation of the first true leaves. The pathogen invades the hypocotyls at or just below the soil line and causes the formation of a lesion. This lesion is first light brown, changing to dark brown, then to black. As the fungus develops in the tissue the infected area collapses and gives rise to a "wire stem" appearance and the seedling may topple over and die. Rhizoctonia sp. invades cotton stems just before or after emergence and disease development depends on the environment. Delay of symptoms can occur, if the environmental conditions favor the growth of the host, and does not favor the growth of the pathogen. Sinclair (120) stated that cotton seedlings infected in mid-April may not show severe symptoms until late May.

Purposes of Study

The apparent increased economic importance of R. solani in the cotton seedling disease complex, and the discovery in recent years of economically feasible chemotherapeutic compounds for plant disease control, it was decided to study the chemotherapeutic activity of Demosan (1,4-dichloro-2,5-dimethoxybenzene) and compare its chemotherapeutic activity with that of Vitavax (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin) and Plantvax (2,4-dihydro-5-carboxanilido-5-methyl-1,4-oxathiin) under both laboratory and greenhouse conditions for effectiveness against R. solani on cotton seedlings. The following points were studied:

1. Verification of reports by E. I. duPont and Co. on claims of systemic quality of Demosan.
2. Determination of systemic movement of Demosan in cotton seedlings to protect hypocotyls from infection by R. solani.
3. In vitro studies were performed to determine if Demosan had direct action on the fungus.
4. Verification of systemic quality of Vitavax and Plantvax in cotton seedlings against R. solani.
5. Comparison of these systemic qualities among the three fungicides to each other.
6. Determination of any effects on cotton seedling hypocotyl tissues by Demosan.
7. Testing for any effects on reducing sugars in cotton seedling hypocotyls treated with Demosan.

LITERATURE REVIEW

Disease may be defined as a harmful deviation from the normal functioning or physiological processes (71, 72). If the pathogen is living, as for example in the case of fungi and bacteria, it may produce symptoms of disease at any time after it enters the host. For this reason infection is considered as commencing after the host is entered by the pathogen, whether symptoms of disease have yet appeared or not (28).

According to Dimond (24): "The objective of plant chemotherapy is the control of plant disease at a low cost in an efficient manner by compounds that act within the plant.

"From the plant pathologist's point of view, no one chemotherapeutant can be useful against all diseases. Diseases vary in the portion of the plant they damage. For control of a systemic disease more is required of a chemotherapeutant than for a localized disease. Some localized diseases present a simpler situation for chemotherapy than others. The diseases yielding first to chemotherapy on a commercial scale are those requiring the fewest critical properties of a chemotherapeutant.

"From the chemist's point of view, the development of a synthetic chemotherapeutant offers a challenge. Compounds must be made that permeate readily into plant cells without injuring them, that translocate readily, that resist detoxification by the plant for a reasonable time, and that are toxic to the pathogen, but not to the host, or

that react in biochemical systems of the host to increase its resistance to infection without undesirable side effects."

Use of systemic compounds in plant disease control is a relatively new science in comparison to the chemotherapy of human diseases. Antibiotics have played a role in systemic control of plant diseases, because of their translocatability and relatively nonphytoxicity, although many antibiotics can be manufactured synthetically on a commercial scale, and a few can be used economically for plant disease control. In order to avoid confusion between the developmental history of antibiotics and synthetic systemic compounds, it would be appropriate to trace the developmental history of each group of compounds separately.

Development of Antibiotics as Chemotherapeutants

The discovery of penicillin by Fleming, in 1929, accelerated the search by plant pathologists for antibiotics for use in the control of plant diseases caused by bacteria, fungi and viruses (71, 72). Weindling (141) observed that antagonistic effects of *Trichoderma lignorum* Pers. ex Fr. (*T. viride*) against the damping-off fungus *Rhizoctonia solani* Kühn. Later, in 1936, Weindling and Emerson (142) were successful in isolating and characterizing a toxic chemical for *T. lignorum* and named it fliotoxin. Brown and Boyle (13) were the first to demonstrate the potential value of antibiotics in plant disease control by showing that penicillin could control the crown gall organism. Cremlyn (15) considered that at the present time the most important antifungal antibiotic is griseofulvin discovered in the mycelium of *Penicillium griseofulvum* by Oxford, Raistrick, and Simonart (98).

This antibiotic has shown considerable activity against a variety of fungi and has practically no phytotoxicity. Brain (12) obtained significant control of Botrytis cinerea Pers. ex Fr. on lettuce and Alternaria solani Sorauer on tomatoes by allowing the antibiotic to be taken up by the roots and translocated. Streptomycin discovered, in 1944, was isolated from Streptomyces griseus Kransby. It showed activity against a broad range of bacterial pathogens both in vitro and in vivo (21). It was reported that streptomycin was systemic when applied to the roots of plants (94). Ark and Alcorn (4) recorded that streptomycin showed good activity against Erwinia amylovora (Burrill) Winslow, the bacterium causing fire blight of pear. Goodman (53) reported that streptomycin had no eradicative potential against this organism. Soon after the discovery of streptomycin actidione was isolated from the same fungus by Whiffen, Bohonos, and Emerson (1943).is chemically known as cyclohexamide. Hamilton and Szkolink (59) reported that soil application of the semicarbazone derivatives of cyclohexamide provided systemic activity against the cherry leaf spot fungus (Coccomyces hiemalis Higgins) in 3-year-old, potted, Montmorency and English Morello cherries. Hamilton, Szkolnik, and Sondheimer (58) found also that foliar spray applications of the semicarbazone derivatives of cycloheximide acted systemically in vivo against C. hiemalis. Hacker and Vaughn (56) reported that this antibiotic induces preinfection resistance to the black stem rust-organism (Puccinia graminis Pers. f. sp. tritici Eriks. & E. Henn) in spring wheat. In these three studies the criterion for systemic activity was the absence of disease symptoms on plants treated with the antibiotic

and inoculated with the pathogen. Shishiyama, Fukutomi, and Akai (115) stated that cyclohexamide was used as a spray fungicide in Japan for controlling the downy mildew pathogen of onion. If the concentration was higher than 10-20 ppm of cycloheximide, injury on leaves showing white or yellow lesions was found and sometimes yield was decreased. By spraying 25 ppm cycloheximide to leaves, the synthesis of both chlorophyll-a and DNA (Ribonucleic acid) was inhibited.

After the discovery of cycloheximide, in 1946, a large number of antibiotics, which act as bacteriocides and fungicides were discovered. A number of tetracycline antibiotics were reported during the period 1948-53, which are produced by a number of different Streptomyces spp. Between the period of 1950-60 more than 40 antibiotics were discovered which contain a conjugated polyene chromophore. This group contains antibiotics which can be exemplified by tetraenes, nystatin, rimocidin and pimaricin. They show activity against a number of fungi and bacteria (21).

Smale, Monttillion, and Pridham (125) showed the systemic activity of the antibiotic phleomycin against bean rust (Uromyces phaseoli typica Arth.) at a concentration as low as 5 ppm.

Edgington (36) found blasticidin S to be systemically active against rice blast, incited by Piricularia oryzae Cav.

Development of Synthetic Systemic Compounds

The interest in controlling plant diseases by the use of synthetic systemic compounds dates back to the report of Fron (50) on the use of 8-hydroxyquinolin against the Dutch elm disease fungus, Ceratostomella ulmi Buisman in France. A mixture of calcium hydroxide, urea, a

salicylate, and an azo dye was proposed by Feldman et al. (46) as a soil drench in the control of the Dutch elm disease. Horsfall and Zentmeyer (74), and Dimond et al. (30) used 8-quinolinol benzoate as a soil drench for the chemotherapeutic treatment of elms infected with the Dutch elm disease organism and obtained a reduction in symptoms. Their purpose also was to antidote the fungus.

Hart and Allision (66) demonstrated the effectiveness of picric acid, p-tolueolsulfonamide, and sodium bromide against the wheat stem rust organism when applied through roots. Strong and Cation (132) were successful in controlling cedar rust galls by painting them with sodium 2,4-dinitro-o-cresylate.

Howard (76) reported the suppression of symptoms caused by Phytophthora cactorum (Leb. & Cohn) Schreet of maple trees by injecting diaminobenzene dihydrochloride. At about the same time Ark (3) controlled the crown gall bacterium Agrobacterium tumefaciens (E. F. Sm. & Town) Conn. by using a mixture of methanol and 2,4-dinitro-o-cresylate (Elgetol). He claimed he obtained eradication of the pathogen by painting this mixture on the gall tissue.

Chemotherapeutic compounds were shown to give control of some plant viruses and plant virus diseases. Stoddard (128) demonstrated that $CaCl_2$, $ZnSO_4$ and sulfanilamide all inactivated the virus X-disease of peach in diseased buds when they were budded onto healthy trees which were treated with these compounds. Ackermann (1) stated that both antimycin and malonic acid, specific inhibitors of respiration, reduced the yields of tobacco mosaic virus in vivo in direct proportion to their effect on respiration. Locke (89) found derivates

of 4-chlorophenoxyacetic acid of value as chemotherapeutic agents against X, Y, and leaf roll viruses when potatoes were treated under field conditions.

McNew and Sundholm (92) while working on the control of early blight of tomato demonstrated that upward translocation of 4-nitrospyrazole can take place within the tomato plant when the lower leaf is immersed in the toxicant solution. They obtained 44 percent control over the nontreated plants within 36 hours.

Crowdy and Wain (16) observed the activity of phenoxycarboxylic acids against the chocolate spot disease of broad beans caused by Botrytis fabae Sardina. They suggested that the incorporation of these compounds increased the host resistance against this disease. Dimond and Davis (29) presented the same explanation while using benzothiazoles and related compounds against Fusarium oxysporum f. lycopersici (Sacc.) S. & H. Davis and Dimond (19) obtained a reduction in disease severity caused by the same fungus on tomato with several growth regulating compounds; 2,4-dichloro-phenoxyacetic acid, a-naphthaleneacetic acid, 2,4,5-triiodobenzoic acid, B-naphthoxy acetic acid, and indole-3-acetic acid. They suggested that these compounds induced changes in the metabolism of the host by (a) formative effects, (b) reduced plant weight, and (c) by a decrease in the reducing sugar content of the host tissue.

Dimond and Chapman (27) and Stoddard (130) demonstrated the ability of 4-chloro-3,5-dimethylphenoxyethanol and 2-norcamphane methanol to eliminate incipient infections by F. o. f. lycopersici on tomatoes and F. o. f. dianthi (Prill. & Del.) Synd. & Hans. on carnations when used as a soil drench.

Davis and Dimond (18) compared a number of synthetic organic compounds for their chemotherapeutic activity against F. o. f. lycopersici on tomato and found no consistent relation between fungitoxicity in vitro and chemotherapeutic activity in vivo. They reported that F. o. f. lycopersici grew equally well on macerated tissue from plants treated with either sodium 2-benzothiazolyl thio-glycolate, a poor fungitoxicant in vitro, or 4-chloro-3,5-dimethoxyphenoxyethanol, a good fungitoxicant in vitro. This indicated that these compounds (a) were not present in the host tissue, (b) did not produce a fungitoxic component through an interaction with the host's biochemical processes; and (c) did not reduce the nutritive value of the tissues to a point where it limited the growth of the pathogen to a greater extent than the nontreated plants (18). From these results Davis and Dimond (18) postulated that the two chemicals increased the resistance of the host to the Fusarium wilt fungus by virtue of their capacity to alter the metabolism of the host.

Livingston (88) screened 179 compounds against the wheat stem rust organism. Hotson (75) observed that some of the sulfa drugs had a marked fungitoxic activity against the wheat stem rust at a concentration as low as 5 lbs per acre. He further observed that control by these sulfa drugs could be counteracted by p-aminobenzoic acid and folic acid, which he concluded were two vitamins required in the metabolism of Puccinia graminis Pers. f. sp. tritici Erick & E. Henn.

Stoddard (131) reported that control of Fusarium spp. and Xanthomonas pelargonii (N. A. Brown) Starr & Burkh., was obtained on geraniums with soil drenches of 250 ppm oxyquinoline sulfate after infection took place.

Sanders and Allison (109) used conidia of Monilinia fructicola (Wint.) Heney in a bioassay to demonstrate the translocation and systemic activity of 2-pyridinethiol-1-oxide (PTO) in the various portions of cucumber plants. He further noted that the toxicant was readily translocated, especially towards the roots. The concentration of PTO decreased as the time interval between the treatment and the bioassay increased, indicating the breakdown of the toxicant by the host.

Stoddard (131) obtained control of Cladosporium cucumerinum Ell. & Arth. with soil applications of captan, maleic hydrazide and q-cyanoethyl-carbazole. Rich (106) obtained protection of corn seedlings against Phytomonas stewartii with 1000 ppm captan. Napier et al. (96) demonstrated that both foliage sprays and root applications of captan protected foliage of broad beans against B. fabae.

It was shown by Kuc, Williams, and Shay (84) that the application of phenylthiourea to the base of apple leaf petioles increased the resistance of the leaves to the apple scab fungus, Venturia inaequalis (Ke. Wrnt.). It is noteworthy that phenylthiourea is an inhibitor of polyphenol oxidase, an enzyme involved in biochemical resistance to plant pathogens. Hacker and Vaughn (57) stated that the foliage application of the semicarbazone and oxime of cycloheximide effectively reduced wheat stem rust infection without serious phytotoxicity.

Systemic activity of sodium dimethylthiocarbamate (NaDDC) was shown by Pluijgers (101). He observed a slight systemic protection by NaDDC against the attack of tomato (C. cucumerinum).

Davis et al. (20) showed the effectiveness of sydnones against wheat rust as well as bean rust diseases. Some compounds of this group are active against wheat rust whereas others are effective against bean rust, thus showing a marked selective activity.

Dekker and Oort (22) demonstrated that 6-azauracil was systemically active against Erysiphe cichoroacearum D. C. on cucumber with either foliage sprays or soil applications. Dekker (21) stated that 6-furfuryl-aminopurine (Kinetin) has also been shown to be inhibitory to the development of the powdery mildew, E. cichoracearum D. C. Dekker and van der Hoek-Schuer (23) observed that certain derivatives of purines and pyrimidines were systemically active against powdery mildew of wheat, E. graminis tritici Em. Marchal. In their experiments they used a number of substituted purines; the most effective among them being 6-azauracil. This compound prevented the formation of haustoria by the fungus.

Joworski and Hoffman (79) tested phenylhydrazones of various aldehydes and ketones against wheat leaf rust, and observed that acrolein phenylhydroaone was the most effective compound with the least phytotoxicity. This further substantiated the concept that the phenylhydrazine is the actual toxicant. MacLennan, Kuc, and Williams (90) reported the inhibition of the apple scab disease caused by Venturia inaequalis by αC-amino-isobutyric acid when infused into leaves at a concentration as low as 0.03 M. αC-aminoisobutyric acid did not inhibit the growth of the fungus in vitro even at 0.40 M concentration. The authors concluded from their experiments that αC-aminoisobutyric acid alters the host metabolism so that resistance to the attach of the apple scab fungus is developed.

Heyns et al. (69) demonstrated that certain derivatives of N-carboxymethyl dithiocarbamic acid, when applied to the roots, had a chemotherapeutic effect against some disease-causing organisms. Some of the derivatives showed systemic protective activity against Erysiphe graminis DC. on pea.

Rapid development of organic fluorine chemistry has taken place in the last 25 years (83), but few fluorine compounds have been explored for possible use in plant protection. Finger, Reed, and Tehon (48) screened a number of aliphatic as well as aromatic fluorine compounds for fungicidal activity but they did not discuss the possibility of their systemic activity. Van Andel (132) showed the systemic activity of fluorophenylalanine against C. cucumerinum and Colletotrichum lagenerium (Pass) Ell. & Halst.

Rapid development of haloid chemistry has taken place in the last 25 years and new chlorine compounds have been explored for possible use in plant chemotherapy (83). Allen and Freiburg (2) recorded the systemic activity of symmetrical dichlorotetrafluoroacetone (DCTFA) against a number of rust fungi attacking different hosts. They obtained control of Uromyces phaseoli typica Arth. on pinto beans and Puccinia recondida Rob. (race 11) on wheat when applied to the soil prior to inoculation. George (52) used the hydrate of DCTFA as a spray in field tests and obtained a 50 percent increase in grain yield of wheat and a 70 percent increase in sheaf weight in comparison to the control. Recently Hardison and Anderson (65) showed the effectiveness of DCTFA against the established rust infections of leaf rust, stripe rust and partial control of stem rust on Kentucky bluegrass. Sinclair

and Darrag (121) reported control of Rhizoctonia solani on cotton seedlings after either seed treatment or soil applications of 1,4-dichloro-2,5-dimethoxybenzene-1823 (Demosan). Fielding and Rhodes (47) demonstrated that Demosan concentrated in the roots and lower stem portions in cotton and bean plants when it was applied to the soil. Maier (91) reported that Demosan was very good for the control of R. solani (=Thanatephorus cucumeris). Bioassays with Demosan incorporated into agar media indicated significant growth suppression of R. solani at 4-8 ppm, with no growth at 125 ppm. Maier (91) also stated that Demosan can be taken up by cotton seedlings and accumulated slightly in their stems, with period of protection being about 3 weeks.

El-Zayat, Lukens, and Horsfall (45) reported that several nitrophenols reduced sporulation of Alternaria solani, and were found to control Erysiphe polygoni on Phaseolus vulgaris L. They indicated when potted plants were watered from the bottom with 125 ppm of 2-chloro-4-diisobutyl-6-nitrophenol (CDNP), they were protected from mildew. CDNP (500 ppm) applied to one primary leaf protected the opposite primary leaf; applied to both primary leaves, it protected subsequent secondary leaves; and applied to secondary leaves, it protected the primary leaves. None of the other nitrophenols had the degree of systemic activity shown by CDNP. The CDNP treatments did not injure the beans (45).

Pellegrini, Bugiani, and Tenerini (99) showed, by means of bioassays and autoradiography, the systemic properties of compounds belonging to the class of B-amino-arylethyl-ketones. They also showed that these compounds exhibited good control against Uromyces

appendiculatus (Pers.) Unger and Plasmopara viticola (Berk.) & Curt.) Berl. & Detoni when applied to the roots of the test plants.

Tempel and Sijpesteijn (135) obtained complete control of Sphaerotheca fuliginea (Schlecht. ex. Fr.) Poll on cucumber seedlings using root applications of phenobarbitol and its sodium salt at 30 ppm.

Recently, several new experimental systemic chemotherapeutants were released by various commercial companies for study. The E. I. duPont de Nemours and Company released a compound by the code number of 1991. The chemical composition is still confidential at this writing. Preliminary testing showed the compound to be systemic in plants and to have "preventative, residual and curative effects" on a wide range of fungi and some species of mites. Maier (91) evaluated the effectiveness of duPont 1991 as a drench in greenhouse flat experiments and as an in-furrow spray in the field in small plot tests. In the greenhouse, duPont 1991 gave better stands (plant survival) at 4 lbs/acre (active) than at 1 lb/acre in R. solani infested soil.

The compound "Thiabenzdazole" (TBZ), released by Merck and Company, was reported by the company not only to be taken up by the roots of certain plants and to move systemically, but also to move from leaf to leaf. This movement in the above-ground parts is apparently unique. Some of the imperfect fungi, as well as Ascomycetes, excluding yeasts, are sensitive to TBZ.

Thompson-Hayward Chemical Company released TH7462, a systemic fungicide active against the powdery mildew fungi on several hosts.

The U. S. Rubber Company released several new experimental compounds. The code numbers of these compounds are: D735 (Vitavax),

F461 (Plantvax), and F849. D735 and F461 were tested in the studies of this thesis.

Edgington et al. (41) demonstrated Vitavax and its sulfone analog Plantvax to be highly selective for most Basidiomycetes. It was shown that these compounds were especially effective against the organisms causing rusts and smuts (42, 63) as well as against species of Rhizoctonia in vitro (11, 111, 122, 124, 139).

Much work has been done using these compounds as seed treatments for the control of various rust organisms. Vaughn et al. (138) and von Schmeling and Kulka (139) showed these compounds to control bean rust, incited by Uromyces phaseoli typica Arth. Powekon and Shanier (102), and Hardison (63) showed these compounds to control wheat stripe rust incited by Puccinia striiformis West. Control of wheat leaf rust, incited by Puccinia rubigo-vera tritici (Eriks.) Carleton also was obtained (139). Edgington and Corke (38) stated that Vitavax was an excellent chemotherapeutant for certain rust diseases when applied to the soil just prior to inoculation of plants. The control of various smut-causing organisms was also reported for these compounds. Browning and Lambe (14) obtained control of loose smut of oats, incited by Ustilago avenae (Pers.) Rostr. Loose smut of barley, incited by U. nuda (Jens.) Rostr., was controlled with the use of these compounds (40, 42, 68, 82). These compounds also were shown to control loose smut of wheat, incited by U. tritici (Pers.) Rostr. (44, 60, 61). Edgington and Kelly (39) obtained control of the onion smut organism, Urocistis apulae Frost, using Vitavax and Plantvax. Hardison (64) stated that stripe smut (Ustilago stiiformis) has been controlled in infected grain

plants by root absorption of two systemic fungicides (derivatives of 1,4-oxathiin): 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4, 4-dioxide (DCMOD), and 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (DCMO). DCMO was much less effective than DCMOD. DCMOD represents an effective chemical for suppression of U. striiformis in infected grain plants and may provide a practical control for lawn and turf diseases.

Preliminary studies by von Schmeling and Kulka (139) showed Vitavax and Plantvax to be active against species of Rhizoctonia in vivo. Schultz (111), using Vitavax, obtained good control of Rhizoctonia sprout necrosis during early phases of growth of Irish potatoes.

Sinclair et al. (124) obtained good control of cotton seedling damping-off in the field using Vitavax as a soil treatment. Sinclair, Darrag, and Borum (122) showed that Vitatax gave good control of R. solani on cotton seedling hypocotyls under greenhouse conditions as a soil treatment. Borum and Sinclair (11) obtained good control of R. solani in laboratory and greenhouse studies using Vitavax as both a seed and soil treatment.

The Modes of Actions of Chemotherapeutic Compound in Plants

Dimond stated that: "Chemotherapy is the control of disease by compounds that act from within the plant. The most obvious type of chemotherapy, and the best known, is the direct action of a compound on the pathogen. Many studies have taken this direct approach and sought compounds that are toxic to the pathogen in vitro, low in phytotoxicity, and sufficiently systemic and stable in the host to be useful."

Dimond (25) classed chemotherapeutic agents into three general categories, according to their mode of action: (1) systemic fungicides and bactericides, which act directly upon the pathogen; (2) compounds that act upon the host itself, causing it to become more resistant to disease, and (3) a group of chemotherapeutants whose action is not known.

Systemic Fungicides and Bactericides

The first of Dimond's (25) three categories included systemic fungicides and bactericides that are directly toxic to the pathogen or become toxic after modification in the plants. He stated that a compound must be shown not to be modified in the plant and be present in toxic concentrations if it is to be considered to act directly. He pointed out that this criteria was fulfilled by the work of Crowdy, Grove, and Pramer (17) who identified streptomycin chromatographically in treated plants in concentrations toxic to the pathogen, and by Pramer, Robinson, and Starkey (103) who used two strains of Erwinia chrysanthemi, one susceptible and the other resistant to streptomycin as a means to demonstrate the control of bacterial infection in streptomycin-treated chrysanthemum cuttings.

Nickel compounds used in combating cereal rust fungi is another example of direct action. According to Dimond (25), Forsyth showed that the nickelous ion and sulfadiazine inhibited respiration and development of Puccinia recondita in wheat. Many years ago Gassner and Hassebrauk, as cited by Dimond (25), reported the chemotherapeutic effect of sulfa against the wheat rust fungus in greenhouse tests, but could not reproduce the effect in the field. Hardison (62) reported

that nickel sulfate gave almost perfect control of the stripe and leaf rust fungi in bluegrass.

Dimond (25, 26) further pointed out that: "A chemotherapeutic compound may be modified in the plant to a form that is fungitoxic. In this case in vitro toxicity of the chemotherapeutic compound may be low, but fungitoxic materials can be isolated from the treated plant." An example of this type of action was found by Lemin and Magee (86) who showed cycloheximide acetate not to be fungitoxic, but that fungitoxic materials were found in plants treated with this antibiotic. They concluded that the acetate was hydrolyzed in plant tissues, thus freeing cycloheximide (25).

Chemotherapeutic compounds may be modified so that the toxic portion of the molecule is masked. Such masking of fungitoxic groupings can be useful in chemotherapy. When masking prevents liberation of a fungitoxic group in host tissue, except in the presence of the pathogen, the chemotherapeutic compound will have a longer, useful life in the host and may show selectively toxicity (25, 26).

Edgington (33, 35) investigated the relation between molecular structure, fungitoxicity, and mobility in plant stems of quaternary ammonium compounds. He found that fungitoxicity decreased as the aliphatic chain length decreased, and that mobility of these compounds increased simultaneously. Thus, it appeared that the best compromise between mobility and fungitoxicity for therapy of certain diseases consisted of a quaternary ammonium compound with a side chain of 6 to 8 carbon atoms.

Dimond (25) suggested that: "An alternate approach to this distributional problem involves suppressing the ionization of the

quaternary ammonium ion in a nonphytotoxic organic solvent that will penetrate through overlying tissues and carry the chemotherapeutic agent into xylem. Then, in the presence of the transpiration stream, the agent regains its ionic charge and is adsorbed to xylem tissue. This approach is also an exploitable one, if a suitable solvent system can be developed."

Compounds that Modify the Host

Dimond (25) divided the second of his three categories into five considerations:

(a) Agents that modify carbohydrate levels in plant tissue. Horsfall and Dimond (71, 71) speculated that it should be possible to increase the resistance of the host to a pathogen and evidence of this was provided by Davis and Dimond (18, 19) who showed that some chemotherapeutants produce morphological and biochemical modifications in the host. One of these biochemical changes may be the increase in concentration of reducing sugar. They postulated that the chemicals increased the resistance to the Fusarium wilt pathogen in tomato by virtue of their capacity to alter the metabolism of the host. This suggested that sugars were important in wilt and that therapeutants may affect sugar concentration, and hence, resistance. Horsfall and Dimond (73) stated that: "quite apart from the effect of nutrient elements in affecting resistance to disease, resistance and susceptibility were related to the sugar content of plant."

Certain pathogens may be classified as high sugar organisms, that is, encouraged by high sugar content of the host, other pathogens may be classified as low sugar organisms because they are favored by

a low sugar content in the host (25, 28, 77). The organisms that cause rusts, powdery mildews, and chocolate spot of broad bean are favored by high sugar content in host cells (25, 77). Guinn and Hunter (54) and Guinn and Stewart (55) reported that sugars accumulated in cotton seedlings when the temperature was lowered. Sugar content of leaves was twice as much when the roots were chilled at 15°C as it was in leaves of plants whose roots were kept at 30°C. They noted more than a 4-fold increase in sugar contents of epicotyls, hypocotyls, and roots in response to a low root temperature. Chilling roots caused a rapid increase in sugar content of stems, reducing sugars doubled and nonreducing sugars increased about 7-fold in two days. They found that homogenate from chilled stems supported almost twice as much growth as seedling disease fungus, R. solani, as did the homogenate from unchilled plants. A relationship between sugar content and disease susceptibility in chilled cotton seedling was suggested. Alternaria sp. on tomato, Helminthosporium sp. on cereals and Ceratostomella ulmi are "low sugar diseases," i.e., tissues low in sugar are attacked (25, 77).

Dimond (25) used as other examples, the following:

"Light, boron deficiency, and the action of growth regulators, such as 2,4-D and maleic hydrazide, and of fungicides such as nabam and captan, all affect the sugar levels in plant tissues (25, 77). In turn, these compounds cause plants to become more susceptible to some pathogens and more resistant to others.

"Diseases caused by organisms that favored by a high sugar content are reduced by 2,4-D and increased by maleic hydrazide. The

opposite effect is achieved when working with diseases caused by organisms favored by low sugar content in host cells" (77).

Dimond (25) stated that "The pectolytic and cellulolytic enzymes are frequently important in pathogenesis and some pathogens produce them as adaptive enzymes. Consequently, he concluded that tissues high in sugars will resist invasion by such pathogens and when such fungal enzymes are constitutive, the presence of sugar in host tissue will have no effect.

"Keyworth and Dimond (80) noted that reducing sugar levels were markedly increased, while certain nutrient elements were present in smaller amounts in plants with injured roots. They suggested that the effects of root injury was to alter the metabolism of the host in such a fashion that it becomes more resistant to disease.

"The sugar content of tissues is a useful index, whether or not it determines resistance or susceptibility as such. This relation offers a useful approach to plant chemotherapy. Also, it may yield important information on the biochemistry of pathogenesis"(25, 73).

(b) Agents that modify the morphology of host tissues (25). "In woody plants, resistance to vascular wilt-diseases may be modified through morphological changes that restrict the invasion of the pathogen. Banfield (8) noted the poor ability of the pathogen causing Dutch elm disease to penetrate cell walls. Young elms with thick annual rings frequently recover from the disease by outgrowing the fungus, whereas old trees with thin annual rings seldom do. When a tree grows rapidly, it merely leaves the pathogen behind, and newly developed tissues are healthy.

"This principle has been considered in chemotherapy of wilt diseases. The critical consideration is to increase the amount of wall substance that the pathogen must penetrate to keep pace with the growth of the tree. In principle, resistance can result from increasing the diametric rate of growth, as happens naturally in young trees as compared with old ones, or it can result from altering the nature of the growth so that fewer vessels are formed, especially the large, early vessels.

"Edgington (34) has markedly reduced the symptoms of Dutch elm disease in inoculated trees with mixed isomers of aminotrichlorophenyl acetic acid. When applied in early spring, this preparation modifies the structure of the woody tissues that develop subsequently in a striking way. A layer of dense, starch-filled cells interrupt the normal continuity of the annual ring. This layer apparently acts as a barricade which the pathogen does not penetrate readily.

"Smalley (126) working with the related, but somewhat more phytotoxic, 2,3,6-trichlorophenylacetic acid has reported its efficacy in preventing Dutch elm disease. This compound also modified growth of the plant and is also more effective when applied early in the growing season. Smalley has noted the ready development of tyloses in vessels of treated trees, and has called attention to the possibility of their acting as an internal barricade in a vessel. The tyloses may function in preventing longitudinal invasion of the tree by the pathogen. That tylose and gum formation can act as an effective barrier to invasion of vascular pathogens has been effectively demonstrated by Beckman, Halmos, and Mace (9)."

(c) Agents that modify amino acid metabolism of the host (25). "Certain unnaturally occurring amino acids increase the resistance of plants to disease. Amino acids that act in this way affect auxin action. The fundamental basis of their activity as chemotherapeutic agents is unknown" (25, 136).

(d) Agents that may influence the phenolic composition (25). "Inhibitors of polyphenol oxidase and precursors of phenolic compounds that function in natural biochemical resistance have exerted moderate activity in preventing Venturia infection on apple leaves and Cladosporium investion on cucumber seedlings" (25, 84).

(e) Agents that modify the pectins in host tissues (25). "A variety of compounds that have growth regulating activity increases the resistance of plants to disease. These compounds apparently affect the nature of pectins in plant tissues, so that they are more resistant to attack by pectolytic fungal enzymes" (29, 37).

MATERIALS AND METHODS

The Test Fungus

The first report of a disease incited by Rhizoctonia sp. was made by Duhamel, in 1728 (32, 100), who described the fungus on Saffron (Crocus sativus L.), Persoon, in 1801; regarding the fungus as a sterile form, he placed it in the genus Sclerotium. DeCandole, in 1815, created the genus Rhizoctonia to accommodate the fungus on Saffron, R. crocorum (Pers.) DC. (140). Kühn (32), in 1858, described a new species on potato, which he named R. solani Kühn.

The relation of R. solani to the Basidiomycetes was established early in the 20th Century by Patouillard, in France, who was the first to describe the basidial stage as Hypochnus filamentosus, in 1891 (140). Prilleux and Delacroix described it, in the same year, on potato stems and named it Hypochnus solani (140). Rogers classified the organism as Pellicularia filamentosa (Pat.) Rogers, in 1943 (140).

Recently, Talbot (133) considered the perfect stage of R. solani to be Thanatephorus cucumeris (Frank) Donk (basionym Hypochnus Frank). Hypochnus solani Prill. & DeLacr and H. filamentosus Pat. are considered to be synonyms of T. cucumeris (133). Talbot (133) pointed out that the generic name of Pellicularia was rejected as being nomenclaturally invalid because: (1) Botryobasidium and Ceratobasidium were readily differentiated from Thanatephorus, while Corticium and Hypochnus were not acceptable for taxonomic; and (2) of certain nomenclatural reasons (133).

T. cucumeris is now regarded as a collective species which includes both the imperfect stage, R. solani, and the perfect stage P. filamentosa. The fungus will be referred to throughout this report by the name of its imperfect stage R. solani.

Isolate T of R. solani was used throughout this study. Isolate T was isolated by Sinclair (116), in 1957, from diseased cotton seedlings collected in the Mississippi River Delta near Roosevelt, Louisiana. This isolate was shown to be highly pathogenic to cotton (116). It was identified as R. solani by Sinclair (116) and verified by other workers both in this laboratory and at the University of California, Berkeley. Isolate T was used in this laboratory for various studies, including: (a) evaluation of soil fungicide (116, 117, 118, 119); (b) the mode of penetration into cotton seedling hypocotyls (81, 123); (c) the nuclear phenomena and chromosome number (112); (d) the ultrastructure of the vegetative mycelium (113); and (e) the systemic fungicides for the control of cotton soreshin (10, 121, 122). A culture is on deposit in the American Type Culture Collection.

Cottonseed Sources

Three varieties of machine- and acid-delinted Upland cottonseed (Gossypium spp.) were used in the laboratory and greenhouse experiments. The cottonseed samples of the varieties Deltapine 15 and Deltapine Smoothleaf were provided by the Delta and Pine Land Company, Scott, Mississippi. The cottonseed sample of the variety Stonevile 213, was provided by the U. S. Rubber Company, Bethany, Connecticut. Machine-delinted seed will be referred to by "MD" and acid-delinted seed by "AD" throughout this dissertation.

Test Chemicals

Three chemical compounds were used:

Demosan (E. I. duPont de Nemours and Co., 1823)-1,4-dichloro-2,5-dimethoxybenzene;

Vitavax (U. S. Rubber Co., D735)-2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin; and

Plantvax (U. S. Rubber Co., F849)-2,4-dihydro-5-carboxyanilido-5-methyl-1,4-oxathiin.

The 75% wettable powder formulation of Demosan was used in all the laboratory and greenhouse experiments except in a single flask-technique experiment in which 90% technical Demosan was used. The label names of these compounds will be used throughout this dissertation.

In the present investigation, laboratory and greenhouse experiments were conducted to: (a) develop techniques for evaluating various chemicals for their systemic chemotherapeutic value against R. solani; (b) determine if any physical changes occurred in host tissues treated with Demosan; and (c) determine if there was a correlation between the reducing sugar content in cotton seedlings treated with Demosan and susceptibility to the test fungus.

At the beginning of this research program, there were no published works on the use of systemic fungicides for the protection of cotton seedlings against infection by R. solani. Systemic activity was reported by several industrial firms in other crops. Special techniques had to be developed for determining the systemic chemotherapeutic protection of fungicidal compounds in cotton seedlings.

Both laboratory and greenhouse techniques were developed to test different fungicides for their systemic activity in cotton seedling hypocotyls against R. solani.

Development of Techniques Used for Evaluating Chemicals for Systemic Chemotherapeutic Activity Against R. solani in Cotton Seedlings

I. Laboratory Techniques

MD cottonseed of the varieties Deltapine 15 and Deltapine Smoothleaf were used.

Inoculum for the laboratory studies were prepared by growing cultures of R. solani, isolate T, on potato-dextrose-agar (PDA) for 4-6 days. A sterile #4 corkborer was used to cut out agar discs containing the fungus from these cultures. Each disc was 0.8 cm in diameter and was used both to seed other PDA plates and flasks containing potato-dextrose-broth (PDB). Discs from the stock culture plates also served as an inoculum source for infection of cotton seedlings.

PDA and PDB were prepared using standard methods. Two techniques were developed for laboratory evaluation of the activity of systemic fungicides.

The Glass Dish Technique. Large specimen dishes (17.5 cm wide by 6.5 cm deep) were sterilized using mercuric bichloride (1:1000) and rinsed with sterile distilled water. Approximately 1500 ml of autoclaved vermiculite (Terralite Brand) was placed in each dish. A polyethylene disk was cut slightly larger than the diameter of the dish and placed on top of the vermiculite. Twenty holes were cut in the disc with a sterile #4 corkborer.

A typical experiment consisted of four treatments: (a) no fungus, no fungicide; (b) no fungus, with fungicide; (c) with fungus, no fungicide; and (d) with fungus, with fungicide. The fungicide was used at the rate of 600 ppm (0.16 grams of Demosan + 200 cc PDB). PDB was used as a nutrient solution in all dishes. Fungicide and broth were mixed before adding to the vermiculite. Check dishes had broth added, without fungicide. This technique was tried as pilot experiment, so one dish was used for each treatment, and 20 surface-sterilized delinted cottonseed of the variety Deltapine 15 were used per treatment. Infested dishes were inoculated with 15 discs of the test fungus. Seed was planted by one of three variations as follows:

Variation 1: Cottonseed were planted into the polyethylene disc holes (one seed per hole) and then covered with a thin layer of sterilized vermiculite at time of planting.

Variation 2: Cottonseed were germinated first in sterilized vermiculite and after four days, a single germinated seed was transplanted into each hole in the polyethylene disc and then covered with a thin layer of sterilized vermiculite.

Variation 3: A single cottonseed was planted into each hole in the polyethylene disc and after most of them germinated, they were covered with a thin layer of sterilized vermiculite.

For all three methods fungus discs were placed above the polyethylene disc between the seed before covering with the vermiculite. The seed and the polyethylene discs were surface-sterilized with mercuric chloride 1:1000 for 5 min and then rinsed three times with sterilized, distilled water.

The number of emerged seedlings was recorded after one week. The results from the pilot experiment are presented in Tables 4, 5, and 6. It appeared that variation 1 was the best and was used in all experiments for testing fungicide in the laboratory.

The Flask Technique. This technique was developed for use in the laboratory, using 125 ml and 250 ml Erlenmeyer flasks, to determine the systemic chemotherapeutic activity of Demosan. To aid in the selection of uniform, healthy seedlings for each experiment, cottonseed were germinated in sterilized, large specimen dishes. Approximately 1500 ml of sterile vermiculite was placed into each dish. Several hundred, surface-sterilized cottonseed were sown on top of the vermiculite. The cottonseed were surface-sterilized for 5 min in mercury bichloride (1:1000) and then washed in sterile, distilled water. A thin layer of autoclaved vermiculite was placed over the seed and then moistened with sterile, distilled water. The dishes were kept in continuous light at room temperature (22-27 C). Fungicide stock solutions were prepared by adding to 1000 ml of sterile, distilled water, Hoagland's solution, or PDB either 0.0, 0.1333, 0.3999, 0.7998, 1.1997 or 1.5996 gm of Demosan 75% wettable powder or 0.0, 0.1111, 0.3333, 0.6666, 0.9999 or 1.3332 gm of Demosan technical 90% to give concentrations of approximately 0, 100, 300, 600, 900, and 1200 ppm, respectively. Distilled water, Hoagland's solution, and PDB were autoclaved for 15 min at 15 lbs pressure before adding the fungicide to them. This technique was repeated four times using different nutrient media in order to select the best combination for evaluation (Table 1).

Empty flasks or flasks which contained either vermiculite or soil (Table 1) were autoclaved for 15 min at 15 lbs pressure. Fungicide solutions were poured into each 125 or 250 ml Erlenmeyer flasks. There were as many flasks prepared as needed to provide four replications for each treatment in each experiment. The neck of each flask then was covered with a small piece of plastic wrap (Scott's Cut-Rite branc) and a cup-like depression made in it. The plastic wrap was held in place by a rubber band. The purpose of the plastic wrap was to provide support for the seedlings above the fungicide solution and to keep the inoculum separate from the fungicide solution below. This technique was well suited because it allowed the roots to absorb and translocate the fungicide up into the cotton seedlings and protect the hypocotyls from infection by R. solani. In experiments No. 1 and 2 one small hole was punched in the plastic wrap of each flask with a sterile metal probe, while, four small holes were punched in the plastic wrap of each flask of experiments No. 3 and 4. Seed coats were removed from the selected cotton seedlings. A single germinated seedling was placed in each hole (one seedling per replicate in experiments 1 and 2, and four seedlings per replicate in experiments 3 and 4) so that its roots were immersed in the fungicide solution below. An agar disc containing R. solani was then placed on the plastic wrap in the center between the four seedlings in each replicate in experiments 3 and 4 and beside the seedlings in experiments 1 and 2 so that it touched their hypocotyls. The cup-like depression was then filled with sterile vermiculite covering the agar disc (Plates 1, 2, and 3). This then was moistened with sterile, distilled water. The fungus grew well in the vermiculite under the

Table 1. Key to four flask experiments comparing techniques and different media (Expt. 1 and 2), and rates of Demosan (Expt. 3 and 4) under laboratory conditions.

Expt. No.	Fungicide	Size of flask	Rates in ppm used in each medium	Kind and amount of media in each flask	R. solani
1	Demosan 75% wettable powder	135 ml Erlenmeyer flasks	0 100 300 600	125 cc Hoagland's sol. 125 cc Distilled water 125 cc Potato-dextrose-broth	yes yes yes yes
2	Demosan 75% wettable powder	125 ml Erlenmeyer flasks	0 0 100 300 600	125 cc Hoagland's sol. 125 cc Distilled water 125 cc Vermiculite + 100 cc fungicide sol. 100 cc sterilized soil + 80 cc fungicide sol. 40 cc sterilized soil + 125 cc fungicide sol.	no yes yes yes yes
3	Demosan Technical 90%	250 ml Erlenmeyer flasks	0 0 100 300 600 900 1200	150 cc Vermiculite + 220 cc fungicide sol.	no yes yes yes yes yes yes
4	Demosan 75% wettable powder	250 ml Erlenmeyer flasks	0 0 100 300 600 900 1200	150 cc Vermiculite + 220 cc fungicide sol.	no yes yes yes yes yes yes

conditions of the experiment. The flasks were kept in a room under continuous light at room temperature (22-27 C). The surface of the vermiculite was moistened daily to insure proper growth conditions for the fungus. The number of healthy seedlings was recorded each day for a period mentioned in tables of results.

Histology Studies. Samples were taken at the end of each flask experiment from different treatments to determine if any histological changes were induced by the test fungus and/or the test fungicide in the host tissue. The sections of seedling hypocotyls which came in direct contact with the test fungus were used for study. Hypocotyl samples were cut into approximately 1.0 cm long segments and fixed in Newcomer's solution (97) for at least 36 hours. Without washing in water, the fixed materials were dehydrated by the tertiary butyl alcohol (TBA) method described by Johansen (78). Infiltration and embedding also were carried out in the manner described by Johansen (78) using "Tissuemat" (Fisher Scientific Co.) paraffin with a melting point of 55 C.

Microtome sections were made at 15 microns, fixed on slides with Haupt's adhesive (78) and flattened over a hot plate at 45 C. The slides then were passed through a regular xylol-alcohol series down to water, and stained with an aqueous solution of safranin and fast green (0.5 gm in 100 cc of 95% alcohol) in the manner outlined by Sass (110). All sections were mounted in Canada balsam.

Photomicrograph of all sections were taken using Kodak Plus X film and a Beseler Topcon 35 mm Camera mounted on a Bausch and Lomg phase-contrast microscope.

II. Greenhouse Techniques

The preparation of the inoculum for soil infestation was essentially the same as described by Sinclair (117). Fungus discs were prepared in the manner as previously described. One PDA discs containing the fungus were used to seed 500 ml flasks containing 200 ml of autoclaved PDB. Cultures were incubated for 10 days at room temperature (22 C $\pm$ 5 C). After this period, the broth was decanted off to eliminate any staling products, the fungus mat placed in a Waring blender with 200 ml of sterile, distilled water and blended for about 30 seconds. The resulting mycelial suspension was used to infest the soil for greenhouse studies.

Two techniques were employed in the greenhouse:

a) The Flat Technique; and

b) The Clay-Pot Technique

The Flat Technique. In this technique three treatments, four replicates per treatment, and 41 cottonseed per replicate were used. Large galvanized metal flats (32.5 cm wide by 52.5 cm long by 9.4 cm deep) in which 41 holes were punched for drainage were used. Demosan was used as test fungicide at the level of 1200 ppm (0.32 gm of Demosan + 200 cc tap water). Three treatments were used in this experiment: (1) no fungus, no fungicide; (2) with fungus, with fungicide; and (3) with fungus, no fungicide.

Two flats were used for each replicate. The first flat was filled with nonsterile field soil which was mixed thoroughly with 200 ml of the fungicide solution. Check flats had soil mixed with 200 ml tap water, without fungicide. Then the second flat was placed on the soil surface

of the first one. The purpose of the second flat was to keep the soil containing the fungicide separated from the soil infested with the test fungus. The cottonseed were planted in the second flat by one of four variations as follows:

Variation 1: Forty-one cottonseed were planted in the holes of each flat (one seed per hole) and then covered with 2-inch layer of nonsterilized field soil.

Variation 2: Forty-one cottonseed were planted in the holes of the flat and then were covered with a 1-inch layer of nonsterilized field soil.

Variation 3: Forty-one cottonseed were planted in the holes of the flat and watered. When most of these seed had germinated, they were covered with a 1/2-inch layer of nonsterilized field soil.

Variation 4: Cottonseed were germinated in the laboratory in vermiculite, then 41 selected, germinated seed were placed in the holes of the flat and covered with about 1/2-inch layer of nonsterilized field soil. To the top layer of soil of the second flats of the treatments number 2 and 3 was added 20 ml of the mycelial suspension of R. solani, prepared as previously described. The top layer of soil in all flats was kept moist by daily watering. The percent germination was recorded after 1 week and readings for number of healthy seedlings was recorded after 4 weeks. Results using this technique were not satisfactory, therefore the clay pot technique was developed.

The Clay Pot Technique. Demosan was used as soil treatments to test its ability to control the cotton soreshin disease. The experiment was conducted using sterilized 6-inch, clay pots containing

nonautoclaved field soil. In this experiment four treatments were used as follows: (1) no fungus, no fungicide; (2) with fungis, no fungicide; (3) with fungus, with fungicide; and (4) no fungus, with fungicide.

A set of four pots per treatment, and 20 cottonseed per pot was used. All pots were filled with nonsterilized field soil (approximately 1 1/2 kg in each). An equal amount of tap water (200 cc) without fungicide, was added to the soil of each pot of the treatments number 1 and 2. A Demosan solution of 1200 ppm (0.32 gm/200 cc water) was mixed with the soil of each pot for treatments 3 and 4. After the soil was thoroughly mixed with water or fungicide solution, a sheet of polyethylene was placed over the surface of the soil. The polyethylene sheet had 20 holes punched in it with a #4 corkborer. The cottonseed were planted in the holes with three variations in order to select the best planting method.

Variation 1: A single cottonseed was planted in each hole and all 20 seed were covered with a thin layer of nonsterilized soil.

Variation 2: Cottonseed were germinated in sterilized vermiculite for four days and 20 selected seed were planted in the 20 holes of each polyethylene disc, and covered with a thin layer of nonsterilized soil.

Variation 3: A single cottonseed was planted in each hole and watered. After most of these seed germinated (approximately 7 days from planting), they were covered with a thin layer of nonsterilized soil. To the top layer of soil of four pots of treatments 2 and 3 was added 15 ml of the mycelial suspension of R. solani, prepared as

previously described. This experiment provided four treated-noninfested pots to test phytotoxicity, and four treated-infested pots to test the ability of the fungicide to control Rhizoctonia solani on cotton seedlings. To the four treated-noninfested check pots, and to the four nontreated-noninfested check pots 15 ml of water was added for each. The top layer of soil in all pots was kept moist by daily watering.

The purpose of the polyethylene sheet was to keep the soil containing the fungicide separated from the soil infested with the test fungus. Capillary action might have brought a small amount of the fungicide through the holes in the polyethylene sheet into the infested soil, but this is doubtful. If this did occur, it was considered of little consequence, since fungus mycelium could be seen growing in and on top of the soil about the cotton seedlings in the treated pots.

The number of germinated seed in each pot was recorded after a week, and the number of healthy seedlings in each pot was recorded after four weeks.

The results using the flat technique and the pot technique indicated that the first method of the clay pot technique was the best for testing the ability of the fungicide to control R. solani on cotton seedlings in the greenhouse. Thus, this technique was used in the rest of the greenhouse experiments for evaluating Demosan, Vitavax, and Plantvax for their systemic chemotherapeutic value against R. solani.

Evaluation of Demosan for Systemic Activity in Cotton Seedlings against R. solani

In the laboratory two experiments were conducted using the glass dish technique to determine the systemic activity of Demosan in cotton seedlings against R. solani.

In the first experiment Demosan was incorporated into vermiculite at a rate of 600 ppm (0.16 gm + 200 cc distilled water). These four treatments were tested: (1) no fungus, no fungicide; (2) with fungus, no fungicide; (3) with fungus, with fungicide; and (4) no fungus, with fungicide. The fungicide solution was added to the vermiculite, and the check dishes had equal amounts of sterile, distilled water (200 cc per dish) added, without fungicide. A set of three replicates per treatment, and 20 AD cottonseed of the variety Deltapine 15 per replicate were used. The number of germinated seed was recorded after 10 days, and number of healthy seedlings was recorded after three weeks.

In the second experiment, Demosan as a seed treatment at 9 oz/100 lb was compared with: (1) Demosan incorporated in vermiculite at 600 ppm; and (2) Panogen 15 at the rate of either 2 or 3 oz/100 lb on both AD and MD cottonseed. The experiment had eight treatments (Table 16). Each treatment was replicated three times, and 20 seed were used per replicate. The number of germinated seed was recorded after 10 days and number of healthy seedlings was recorded every 5 days from the tenth day for a period of 30 days.

In the greenhouse, the clay pot technique was used. The same experimental design of laboratory experiments war used except either nonsterile or steam-sterilized soil was used instead of vermiculite. These experiments were repeated twice. Four replicates were used per

treatment, and 15 cottonseed were used per replicate. A reading for number of germinated seed was recorded after 10 days and readings for number of healthy seedlings were recorded every 5 days from the tenth day for a period of 30 days (Tables 17, 18, and 19).

A greenhouse experiment was designed to compare five rates (0.16, 0.32, 0.64, 0.96 gm/pot) of Demosan as a soil treatment including a nontreated check. In this experiment only autoclaved soil was used. The Demosan was added to the soil either 6 days before planting time or at planting time as indicated in Table 2.

Four replications (4 pots) were used per treatment and 15 cottonseed of the variety Deltapine 15 were used per replicate. Number of germinated seed was recorded after 10 days and number of healthy seedlings was recorded every 5 days from the tenth day for a period of 30 days.

Comparison of Vitavax and Plantvax to Demosan for Systemic Activity in Cotton Seedlings against R. solani

Laboratory and greenhouse tests. The glass dish and clay pot techniques were used for this evaluation. Vitavax, Plantvax and Demosan also were compared to Panogen 15 as seed treatments on both acid-delinted and machine-delinted cottonseed. Three experiments were conducted, one in the laboratory and two in the greenhouse. The design of the first two experiments was exactly the same, and in the third experiment Vitavax and Plantvax were compared as soil treatments in the greenhouse at the rate of 0.15 gm of the formula 10% A. I. per pot. In the laboratory experiment each treatment was replicated three times and 20 cottonseed were used per replicate. In the greenhouse experiments eight replicates were used per treatment (four replicates for

Table 2. Key to greenhouse experiment comparing rates of Demosan as soil treatments in the greenhouse.

Treatment combination number	Rate of Demosan in gm	Treatment at Planting	Treatment at Six days after planting	R. solani
1	0		x	no
2	0	x		no
3	0		x	yes
4	0	x		yes
5	.16		x	no
6	.16	x		no
7	.16		x	yes
8	.16	x		yes
9	.32		x	no
10	.32	x		no
11	.32		x	yes
12	.32	x		yes
13	.64		x	no
14	.64	x		no
15	.64		x	yes
16	.64	x		yes
17	.96		x	no
18	.96	x		no
19	.96		x	yes
20	.96	x		yes

the sterilized soil and four replicates for the nonsterilized soil). Each replicate had 15 cottonseed. The design of the three experiments are presented in Table 3. Treatments 1, 2, 17-24 on Deltapine 15 cottonseed was provided by Delta and Pineland Company. Treatments 3 and 4 = Deltapine Smoothleaf provided by the Delta and Pineland Company. Treatments 5-16 on Stoneville 213 was provided by the U. S. Rubber Company.

Number of germinated seedlings was recorded after 10 days and number of healthy seedlings was recorded every 5 days from the tenth day for a period of 30 days.

Bioassay of Demosan-treated Cotton Seedlings

Attempts were made to determine if fungicidal activity could be detected in Demosan-treated cotton seedlings. Two experiments were conducted using cottonseed of the variety Deltapine Smoothleaf.

For the first experiment, the glass dish technique with a single replicate per treatment and 20 cottonseed per replicate was used. Eight concentrations of Demosan in 200 ml water: 0, .133, .266, .399, .799, 1.064, 1.197, or 1.596 gm) to give approximately 0, 100, 200, 600, 800, 900, or 1200 ppm, respectively were compared.

After 7 days, seedlings were removed separately from each treatment and washed with tap water. They were dried gently by pressing them between paper towels. Five gm of tissue from each treatment was surface-sterilized with mercuric chloride (1:1000) for 1 min, then rinsed three times with sterile, distilled water and dried gently by putting them between sterile filter paper.

Table 3. Key to laboratory and greenhouse experiments comparing rates of Demosan, Vitavax, Plantvax and Panogen 15 on various types of seed with and without R. solani.

Treatment No.	Kind of treatment	Fungicide	Rate			Type of seed[1]	R. solani
			Lab. expt.	Greenhouse 1st expt.	Greenhouse 2nd expt.		
1	Vermiculite Treatment only in lab.	Demosan	600 ppm	.32 gm/pot	.32 gm/pot	AD	no
2		Demosan	600 ppm	.32 gm/pot	.32 gm/pot	AD	yes
3	Seed Treatments	Demosan	9 oz/100#	9 oz/100#	9 oz/100#	MD	no
4		Demosan	9 oz/100#	9 oz/100#	9 oz/100#	MD	yes
5		Vitavax	4 oz/100#	4 oz/100#	4 oz/100#	MD	no
6		Vitavax	4 oz/100#	4 oz/100#	4 oz/100#	MD	yes
7		Vitavax	4 oz/100#	4 oz/100#	4 oz/100#	AD	no
8		Vitavax	4 oz/100#	4 oz/100#	4 oz/100#	AD	yes
9		Plantvax	4 oz/100#	4 oz/100#	4 oz/100#	MD	no
10		Plantvax	4 oz/100#	4 oz/100#	4 oz/100#	MD	yes
11		Plantvax	4 oz/100#	4 oz/100#	4 oz/100#	AD	no
12		Plantvax	4 oz/100#	4 oz/100#	4 oz/100#	AD	yes
13		Panogen 15	3 oz/100#	3 oz/100#	3 oz/100#	MD	no
14		Panogen 15	3 oz/100#	3 oz/100#	3 oz/100#	MD	yes
15		Panogen 15	2 oz/100#	2 oz/100#	2 oz/100#	AD	no
16		Panogen 15	2 oz/100#	2 oz/100#	2 oz/100#	AD	yes
17	Soil Treatments	Vitavax			.15 gm/pot	MD	no
18		Vitavax			.15 gm/pot	AD	yes
19		Vitavax			.15 gm/pot	AD	no
20		Vitavax			.15 gm/pot	AD	yes
21		Plantvax			.15 gm/pot	MD	no
22		Plantvax			.15 gm/pot	MD	yes
23		Plantvax			.15 gm/pot	AD	no
24		Plantvax			.15 gm/pot	AD	yes

[1]See text for details on source of seed.

Each tissue sample was ground with 10 ml of sterile distilled water in a sterile motar and pestle. The grindates were filterated into a test tube using sterilized cheesecloth. The tissue extract of each treatment was added to 150 ml of sterile PDA in 250 ml Erlenmeyer flasks. The filterate was added, along with 10 drops of 10% acetic acid, when the agar had cooled to about 50-55 C. The extract was mixed thoroughly with the PDA and 4 plates were poured from each flask. Plates with PDA without extract served as checks.

All plates were seeded by placing an agar disc containing R. sclani in the center of each plate. All plates were incubated at 26 C in an incubator. Inhibition of growth was determined by measuring radial growth of colonies. The diameter of each colony was measured in cm when the mycelial growth on check plates covered the agar (9.0 cm), which occurred at 4 days after seeding.

For the second experiment cottonseed of the variety Deltapine Smoothleaf was germinated in sterilized vermiculite. After three days young seedlings were removed from the vermiculite and rinsed with tap water, then gently dried by pressing them between paper towels. There were seven, 5-gm samples of fresh tissue weighed from these seedlings. Seven large Petri dishes (14 cm) were prepared and contained one of seven different concentrations of Demosan in 200 ml water: 0, 100, 200, 300, 600, 900, or 1200 ppm. The 5-gm samples were placed in the previous fungicide solutions. After 30 hrs exposure, each 5-gm tissue sample was removed from the Petri dish and washed five times in sterilized, distilled water until the water became clear in order to remove any residue of the fungicide from the plants. These were gently dried

by pressing them between sterilized filter paper. Each set was ground separately with 10 ml of melted PDA in a sterilized mortar. The granulates were filtered in separate test tubes using sterilized cheesecloth and the tissue extract added to 140 cc of sterile PDA in 250 Erlenmeyer flasks. The filtrate was mixed thoroughly with the PDA and 4 plates poured from each flask. Plates with PDA, but containing no fungicide, served as checks. All plates were seeded by placing an agar disc containing R. solani in the center of each plate. All plates were incubated at 26 C in an incubator. Inhibition of growth was determined after 4 days as mentioned before.

In Vitro Studies

In vitro studies were conducted to determine if there was a direct action of Demosan upon R. solani.

The method used was essentially the same as that described by Sinclair (119). The fungicide concentration of either 0, 15, 25, 50, 75, 100, 125, or 150 ppm were prepared by weighing 0.0, 0.003, 0.005, 0.01, 0.015, 0.02, 0.025 or 0.03 gm respectively of the 75% wettable powder formulation of Demosan.

The desired quantity of fungicide for each treatment was added to 150 ml of sterile PDA in 250 ml Erlenmeyer flasks. The fungicide was added, along with 10 drops of 10% acetic acid, when the agar had cooled to about 50-55 C. The fungicide was mixed thoroughly with the PDA and four plates poured from each flask. Plates with PDA, but containing no fungicide, served as checks.

All plates were seeded by placing an agar disc containing R. solani in the center of each plate. All plates were incubated at 26 C

in an incubator. Inhibition of growth was determined by measuring radial growth of colonies. The diameter of each colony was measured in cm when the mycelial growth on check plates covered the agar (9.0 cm), which occurred at 4 days after seeding. This experiment was repeated twice. In the first run, the first seven concentrations were used, and in the second run all eight concentrations were used.

Determination of the Effects of Demosan on Reducing Sugars in Cotton Seedlings

Studies were made to determine if Demosan had any effect on the quantity of reducing sugars in cotton seedlings and if any changes in the amount could be correlated to susceptibility to R. solani.

Cottonseed of the variety Deltapine Smoothleaf were used. The glass dish method of planting, inoculation with the fungus, and fungicide application was used. This experiment was repeated twice. Each time the experiment included seven treatments of Demosan (Tables 36 and 37) and each concentration replicated three times. There were 20 cottonseed per replicate. The last six treatments were inoculated with R. solani but the first treatment was left without inoculation. The experiment was kept at room temperature (22-27 C) under continuous light. The experiment was watered as usual in order to insure proper growth conditions for the fungus. An equal number of cotton seedlings were taken at random from each treatment after 7 and 15 days. Seedlings of each treatment were washed separately with tap water to remove any vermiculite attached to them, and excess water removed with a paper towel. Seedlings were dried in the oven at 60 C for 72 hrs. Dried seedlings of each treatment were ground separately using a clean mortar

and pestle. Determination of reducing sugar was repeated three times for each treatment of each experiment. A 0.25 g dried sample of tissue was weighed and put into a 125 ml Erlenmeyer flask to which 50 ml of 80% ethyl alcohol was added. Samples were heated for 10 min in a boiling water bath to destroy any enzymatic activity. Flasks then were allowed to cool to room temperature and were stored in the refrigerator over night. At time of analysis, samples were filtered through Whatman No. 2 filter paper into a 125 ml Erlenmeyer flask. Filtrates were boiled on a hot plate at 80 C to evaporate the alcohol. Ten ml of distilled water was added during this process to prevent drying of the sample. After complete alcohol evaporation, flasks were allowed to cool to room temperature, and samples were treated with 2.5 ml of a saturated solution of neutral lead acetate. Excess lead acetate was removed by adding 5 ml of a saturated disodium phosphate. After the addition of 0.1 g of Norite decolorizing charcoal, the mixture was allowed to stand with frequent shaking for 30 min as mentioned by Forsce (49) and Morell (95). The contents then were filtered through Whatman No. 2 filter paper into a 125 ml volumetric flask. The filtrate was brought up to 20 cc in each flask using distilled water. A 2.0 ml aliquot of this preparation was pipetted into a test tube to which 5 ml of the reagent, alkaline ferricyanide was added. Blanks were included using 2.0 ml of distilled water. The tubes were placed in a wire basket (16 at a time) and set into gently boiling water in such a manner that the contents were immersed to approximately two-thirds of their depth. Heating was maintained for exactly 15 min and the basket was then quickly immersed into cool tap

water. A stream of tap water was run continuously around the tubes for 4 min. The light transmittance of the samples was then determined using photoelectric colorimeter at wavelength 420 mu (Tanimato and Burr (134)). After readings were obtained, the weight in micrograms of reducing sugar in the samples was determined from standard curve made by Rizk (108).

RESULTS

Development of Techniques for Evaluating Chemicals for Systemic Activity against R. solani in Cotton Seedlings

Both laboratory and greenhouse techniques were developed for determining systemic, chemotherapeutic protection by certain fungicidal compounds in cotton seedlings.

Laboratory Techniques

The glass dish and flask techniques were used. The comparison of variations in the glass dish technique indicated that planting variation 1 was the best and therefore was used in all experiments for testing fungicides in the laboratory (Tables 4 and 5).

In comparing variations in the flask technique it was found that the use of vermiculite was better in evaluating disease control by giving a higher percentage of healthy seedlings (Tables 6 and 7, Plates 1 and 2). Therefore the flask technique using vermiculite was used in the laboratory to determine the systemic chemotherapeutic activity of Demosan against R. solani on cotton seedlings (Plate 3). The 75% wettable powder, and 90% technical formulations of Demosan were used in the preparation of test solutions. It was found that the 600 ppm of either formulation gave 100 per cent disease control after 7 days, and 87.5 per cent disease control 10 days after transfer. At 900 and 1200 ppm complete disease control was evident after 7 and 10 days (Tables 8 and 9). All 16 seedlings of the nontreated-noninoculated check flasks remained healthy throughout both experiments. The mean percentage of healthy seedlings in

nontreated-inoculated check flasks decreased during the length of the experiment (Tables 8 and 9). At the end of any one experiment, there were no healthy seedlings remaining in the nontreated-inoculated check flasks (Plate 3).

Slight to moderate phytotoxicity was noted on cotton seedlings at 900 and 1200 ppm, with no evidence of phytotoxicity at 100, 300, and 600 ppm (Tables 8 and 9).

Greenhouse Techniques

The flat and clay pot techniques were used in greenhouse studies. Results obtained using the flat technique were not satisfactory, therefore the clay pot technique was developed (Tables 10 and 11). Results of the clay pot technique indicated that variation 1 was the best for testing fungicidal activity against R. solani, therefore, it was used in all the greenhouse experiments. Variation 1 was easier to set up and at the same time appeared to be more accurate than the other two variations of planting (Tables 12 and 13).

Histology Studies

Samples of hypocotyl tissue were taken at the end of each flask experiment from the different treatments to determine if any histological changes were induced by the test fungus and/or fungicide. Those sections of seedling hypocotyls which came in direct contact with the test fungus were used for this study. Demosan apparently did not alter the physical structure of cotton seedling hypocotyls regardless of concentration used (Plates 4-16). These studies showed that when the concentration of Demosan increased, the protection to the hypocotyl against R. solani increased. Tranverse sections of

Table 4. Per cent of germinated seed (G) and healthy seedlings (H) from three variations in the glass dish technique for evaluating systemic fungicides at one week after sowing.

	Treatments	Variation[1/] 1 G	1 H	2 G	2 H	3 G	3 H
a	No fungus No fungicide	100	100	100	100	50	50
b	No fungus With fungicide	100	100	100	100	65	65
c	With fungus No fungicide	100	0	100	0	55	25
d	With fungus With fungicide	100	100	100	100	55	55

[1/] Variation 1: Cottonseed were planted into the polyethylene disc holes and then covered with vermiculite at time of planting.

Variation 2: Cottonseed were germinated first, and then transplanted into each hole in the polyethylene disc and then covered with vermiculite.

Variation 3: Cottonseed was planted into each hole in the polyethylene disc and after most of them germinated, they were covered with vermiculite.

Table 5. Comparison of the amount of growth of roots and hypocotyls among three variations of the glass dish technique at 3 weeks after sowing.

	Treatments	Variation[1/] 1 Hypocotyl	1 Root	2 Hypocotyl	2 Root	3 Hypocotyl	3 Root
a	No fungus No fungicide	++	++	++	+	++	++
b	No fungus With fungicide	+++	+++	++	++	++	++
c	With fungus No fungicide	-	-	-	-	-	-
d	With fungus With fungicide	+++	++	++	++	++	+

+++ Excellent growth
++ Good growth
+ Fair growth
- Limited growth because of infection

[1/] See Table 4 for details.

Table 6. Mean (4 reps.) per cent of healthy seedlings at 7 days after inoculation using the flask technique comparing different media using the wettable powder formulation of Demosan 75% at rates indicated.

Rate in ppm	R. solani	Kind of media		
		Hoagland's solution	Distilled water	Potato-dextrose broth
0	yes	0	0	0
100	yes	0	25	0
300	yes	50	50	0
600	yes	75	75	0

Table 7. Mean (4 reps.) per cent of healthy seedlings at 8 days after inoculation using the flask technique comparing different media, using the wettable powder formulation of Demosan 75% at rates indicated.

Rate in ppm	R. solani	Kind of media				
		Vermiculite	Soil (40 cc)	Soil (100 cc)	Distilled water	Hoagland's solution
0	no	100	100	100	100	100
0	yes	0	0	0	0	0
100	yes	75	0	25	25	0
300	yes	75	25	25	25	50
600	yes	100	75	50	50	75

Table 8. Mean (4 reps.) per cent of healthy seedlings at 4, 7, and 10 days after inoculation and showing degree of phytotoxicity using the flask technique, with vermiculite and Demosan 90% technical at rates indicated.

Rate in ppm	R. solani	Days after inoculation 4	7	10	Degree of Phytotoxicity[1/]
0	no	100.0	100.0	100.0	0
0	yes	87.5	12.0	0.0	0
100	yes	100.0	93.7	56.2	0
300	yes	100.0	100.0	75.0	0
600	yes	100.0	100.0	87.5	0
900	yes	100.0	100.0	100.0	+
1200	yes	100.0	100.0	100.0	++

[1/] ++ Moderate phytotoxicity
+ Slight phytotoxicity
0 No phytotoxicity

Table 9. Mean (4 reps.) per cent of healthy seedlings from 16 seed at 4, 7, and 10 days after inoculation and showing degree of phytotoxicity using the flask technique with vermiculite, and wettable powder formulation of Demosan 75% at rates indicated.

Rate in ppm	R. solani	Days after inoculation 4	7	10	Degree of Phytotoxicity[1/]
0	no	100.0	100.0	100.0	0
0	yes	62.5	0.0	0.0	0
100	yes	81.2	75.0	75.0	0
300	yes	100.0	81.2	81.2	0
600	yes	100.0	100.0	87.5	0
900	yes	100.0	100.0	100.0	+
1200	yes	100.0	100.0	100.0	++

[1/] ++ Moderate phytotoxicity
+ Slight phytotoxicity
0 No phytotoxicity

Table 10. Comparison of mean (4 reps.) per cent of germinated seed after one week among four variations of the greenhouse flat technique.

Treatments	Variation[1/] 1	2	3	4
1 No fungus No fungicide	1.82	59.75	42.68	79.35
2 With fungus With fungicide	1.21	59.14	36.58	81.09
3 With fungus No fungicide	1.21	64.00	36.58	78.04

[1/] Variation 1: Forty-one cottonseed were planted in the holes of each flat (one seed per hole) and then covered with 2-inch layer of nonsterilized field soil.

Variation 2: Forty-one cottonseed were planted in the holes of the flat and then were covered with 1-inch layer of nonsterilized field soil.

Variation 3: Forty-one cottonseed were planted in the holes of the flats and watered. When most of these seed had germinated, they were covered with 1/2-inch layer of nonsterilized field soil.

Variation 4: Cottonseed were germinated in the laboratory in vermiculite, then 41 selected, germinated seed were placed in the holes of the flats and covered with about 1/2-inch layer of nonsterilized field soil.

Table 11. Comparison of mean (4 reps.) per cent of healthy seedlings after four weeks among four variations of the greenhouse flat technique.

Treatments	Variation[1/] 1	2	3	4
1 No fungus No fungicide	1.82	59.75	42.68	79.26
2 With fungus With fungicide	1.21	57.31	33.53	80.48
3 With fungus No fungicide	1.21	59.14	31.70	74.38

[1/] See Table 10.

Table 12. Comparison of mean (4 reps.) per cent of germinated seed after one week among three variations of the clay pot technique.

Treatments		Variation[1/] 1	2	3
1	No fungus No fungicide	81.25	100.0	78.75
2	With fungus No fungicide	65.00	90.00	80.00
3	With fungus With fungicide	77.50	87.50	73.75
4	No fungus With fungicide	92.50	100.0	85.00

[1/] Variation 1: One cottonseed was planted in each hole and all 20 seed were covered with a thin layer of nonsterilized soil.

Variation 2: Cottonseed were germinated in sterilized vermiculite for 4 days and 20 selected seed were planted in the 20 holes of each polyethylene disc, and covered with a thin layer of nonsterilized soil.

Variation 3: One cottonseed was planted in each hole and watered. After most of these seed germinated they were covered with a thin layer of nonsterilized soil.

Table 13. Comparison of mean (4 reps.) per cent of healthy seedlings after 4 weeks among three variations of the clay pot technique.

Treatments		Variation[1/] 1	2	3
1	No fungus No fungicide	81.25	100.00	78.75
2	With fungus No fungicide	22.50	38.75	50.00
3	With fungus With fungicide	42.50	40.00	47.50
4	No fungus With fungicide	92.50	100.00	80.00

[1/] See Table 12.

Plate 1. Comparison of three rates of 75% wettable powder formulation of Demosan 7 days after transfer using the flask technique with vermiculite. Left to right: nontreated-noninoculated check; noninoculated check, then Demosan at 100, 300, and 600 ppm, respectively. All flasks containing Demosan were inoculated with R. solani.

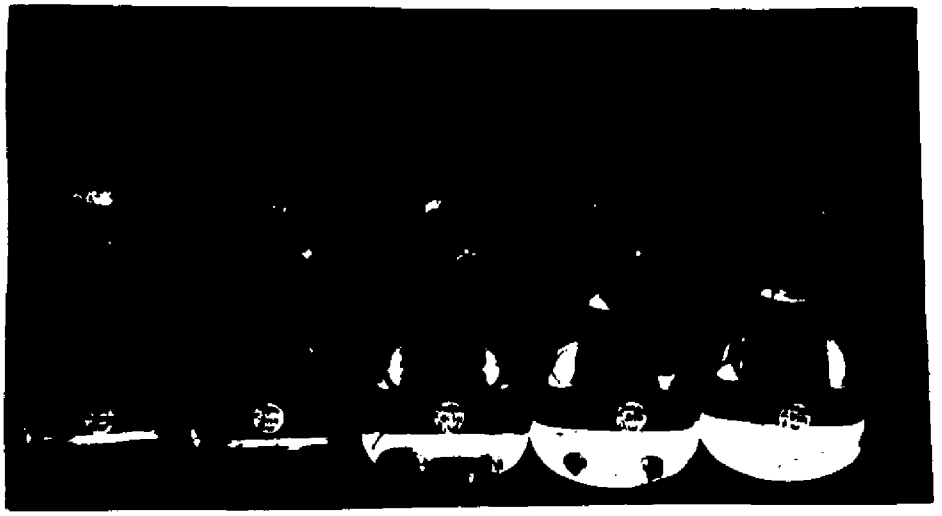

Plate 2. Comparison of three rates of 75% wettable powder formulation of Demosan 7 days after transfer using the flask technique with distilled water. Left to right: nontreated-noninoculated check; nontreated-inoculated check; then Demosan at 100, 300, and 600 ppm, respectively. All flasks containing Demosan were inoculated with R. solani.

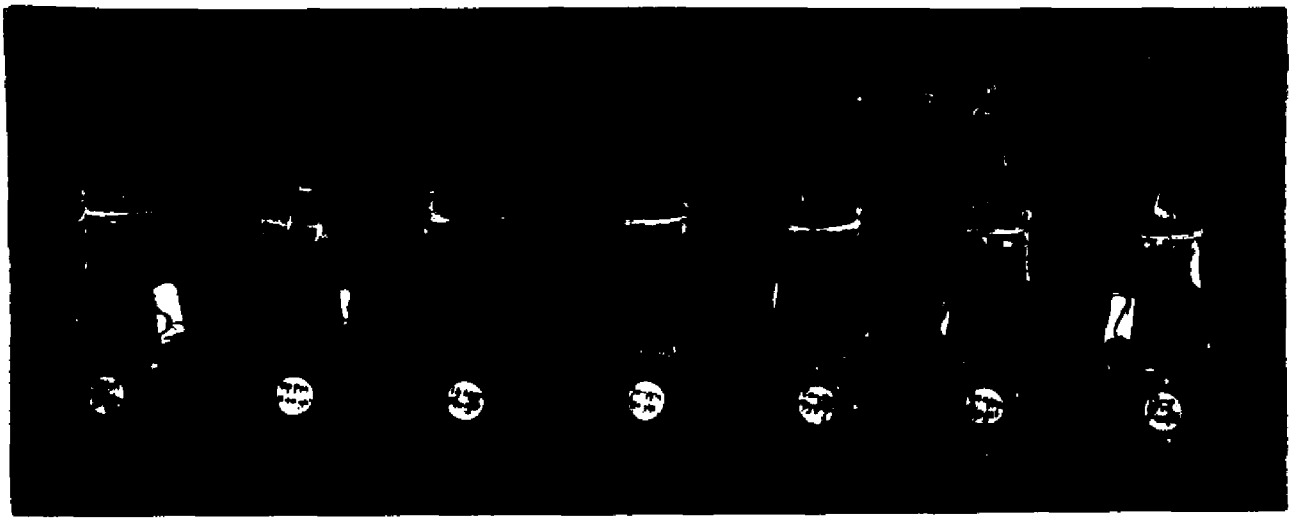

Plate 3. Comparison of five rates of 90% technical formulation of Demosan 8 days after transfer using the flask technique with vermiculite. Left to right: nontreated-noninoculated check; nontreated-inoculated check; then Demosan at 100, 300, 600, 900, and 1200 ppm, respectively. All flasks containing Demosan were inoculated with R. solani.

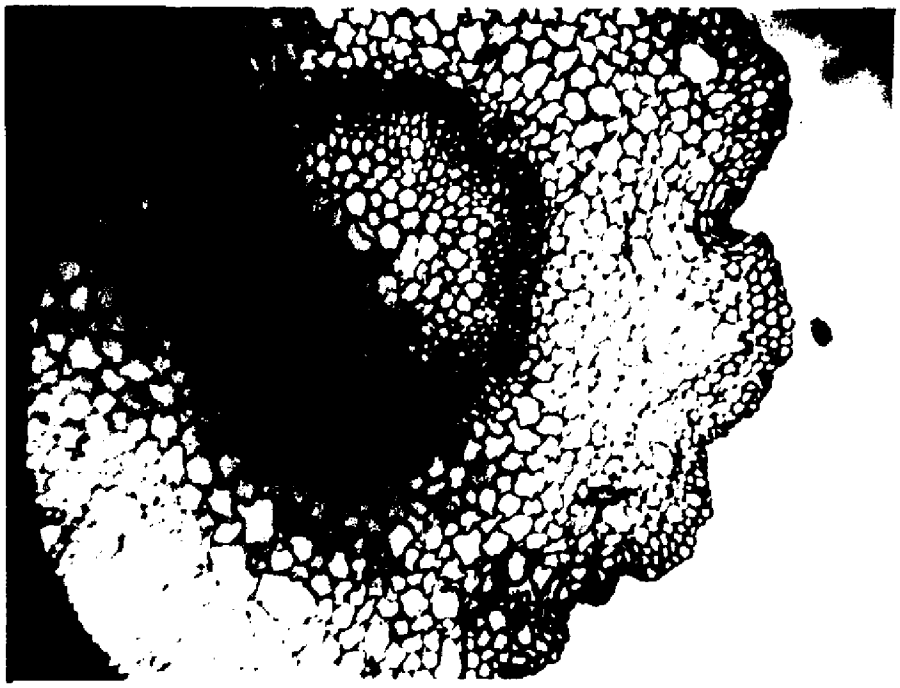

Plate 4. Transverse section of healthy hypocotyl tissue from 10-day-old seedling from nontreated-noninoculated check (X50).

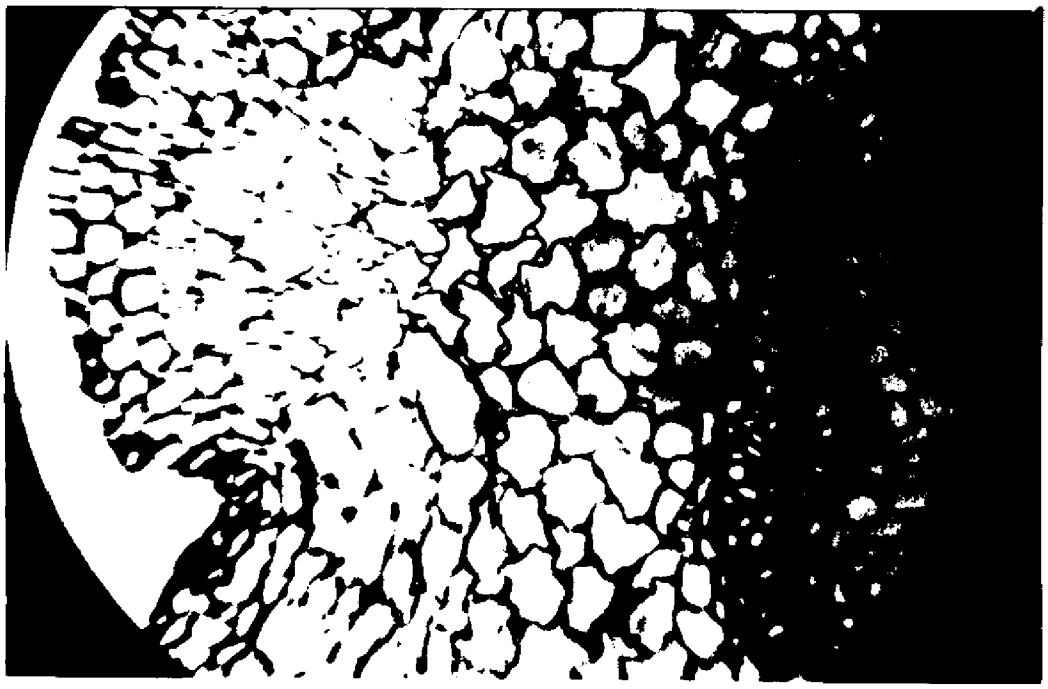

Plate 5. Enlarged area of plate 4 showing healthy hypocotyl tissue from 10-day-old seedling from nontreated-noninoculated check (X210).

Plate 6. Transverse section of infected hypocotyl tissue from 10-day-old seedlings from nontreated-inoculated check showing the invading hyphae (hy) growing through the epidermal cells (ec) to the cortical cells (cc), phloem cells (pc), and through the vascular cylinder (vc) into pith cells (pc). Note the host tissue completely disintegrated up to vascular cells (vc) (X210).

Plate 7. Transverse section of infected hypocotyl tissue from 10-day-old seedlings treated with Demosan at 100 ppm showing the invading hyphae (hy) growing through the epidermal cells (ec) into all the cortical cells in the whole section (X50).

Plate 8. Enlarged area of Plate 7 showing infected hypocotyl tissue from 10-day-old seedlings treated with Demosan at 100 ppm and the invading hyphae (hy) growing through the epidermal cells (ec) into the cortical cells in the whole section (X 210).

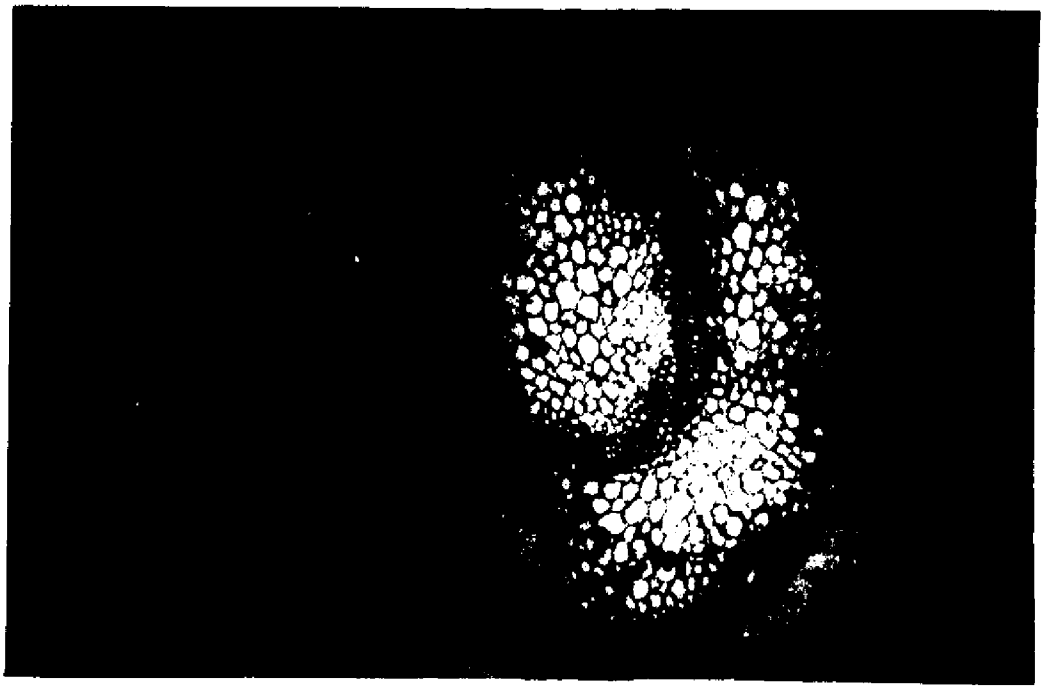

Plate 9. Transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 300 ppm showing the invading hyphae (hy) growing through the epidermal cells (ec) into a few parts of the cortical cells (cc) (X50).

Plate 10. Enlarged area of Plate 9 showing transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 300 ppm and the invading hyphae (hy) growing through the epidermal cells (ec) into a few parts of the cortical cells (cc) (X210).

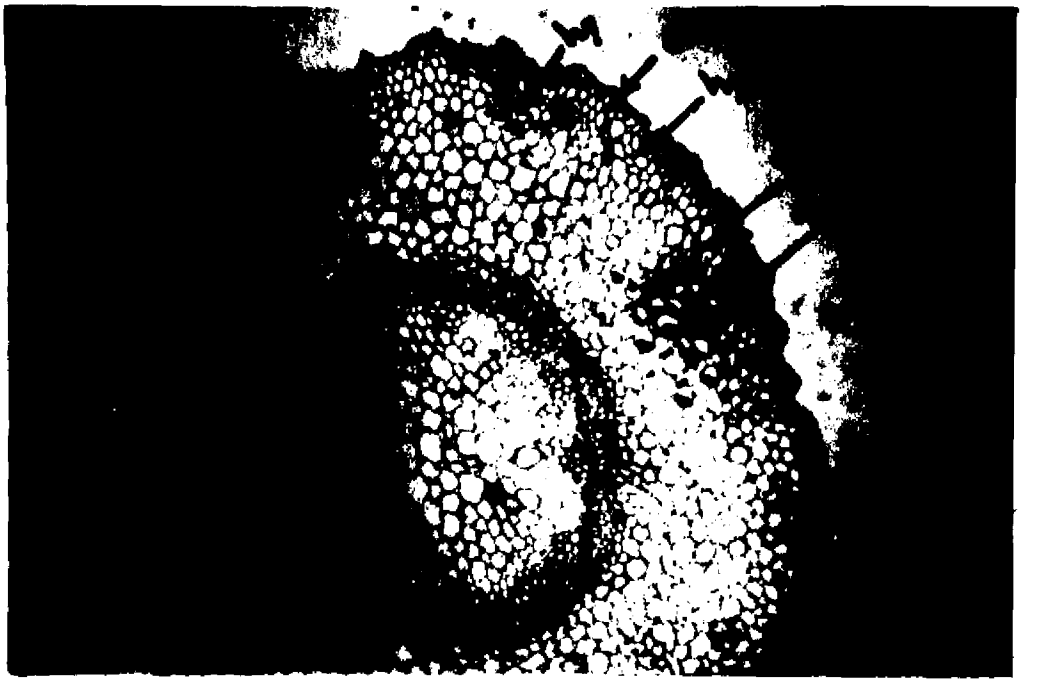

Plate 11. Transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 600 ppm showing hyphae (hy) growing longitudinally in separate parts of the epidermal cells (ec) into a few parts of the cortical cells (cc) (X50).

Plate 12. Enlarged area of Plate 11 showing transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 600 ppm and the hyphae (hy) growing longitudinally in separate parts of the epidermal cells (ec) into a few parts of the cortical cells (cc) (X210).

Plate 13. Transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 900 ppm. Note that the fungus grew on the surface of the epidermal cells (ec) only making dark border (X50).

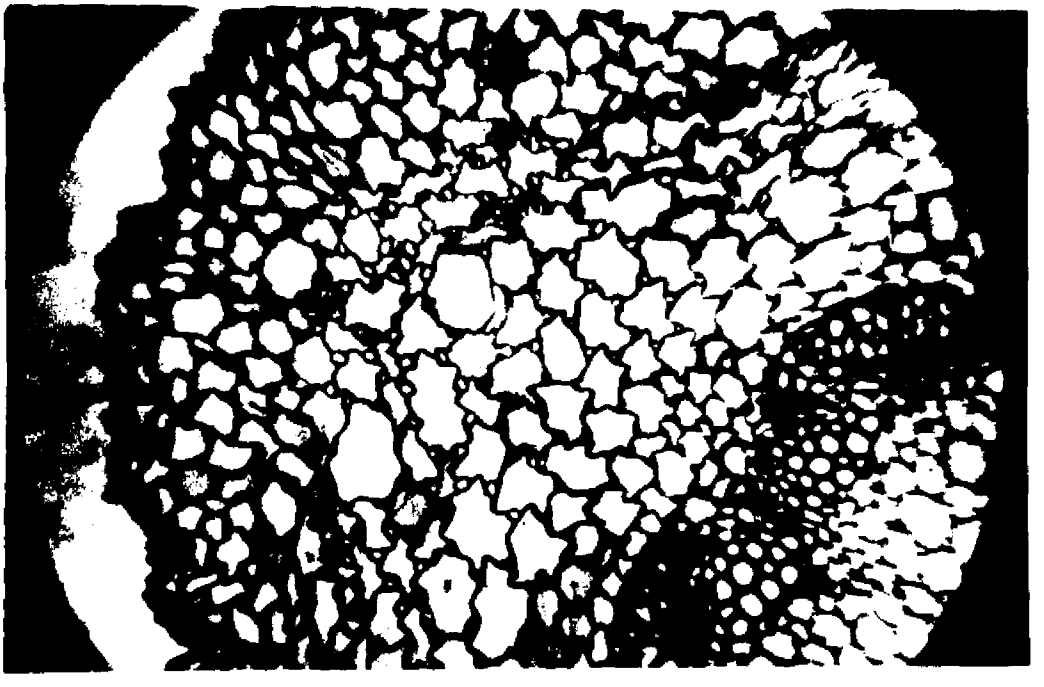

Plate 14. Enlarged area of Plate 13 showing transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 900 ppm. Note that the fungus grew on the surface of the epidermal cells (ec) only making dark border (X210).

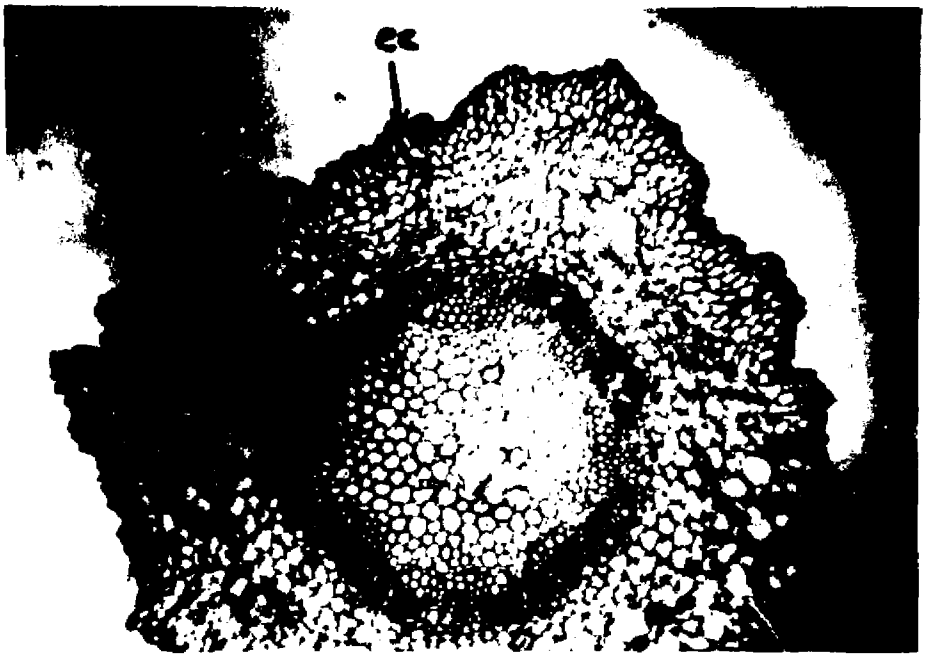

Plate 15. Transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 1200 ppm. Note that the fungus grew on the surface of the epidermal cells (ec) only making dark border (X50).

Plate 16. Enlarged area of Plate 15 showing transverse section of infected hypocotyl tissue from 10-day-old plants treated with Demosan at 1200 ppm. Note that the fungus grew on the surface of the epidermal cells (ec) only making dark border (X210).

infected hypocotyl tissue from nontreated-inoculated checks showed invading hyphae growing through epidermal cells into cortical cells, phloem cells, and through the vascular cylinder into pith cells. The host tissue became completely disintegrated to vascular cells except for xylem tissue which apparently was not attacked (Plate 6). Transverse sections of infected hypocotyl tissue from plants treated with Demosan at 100 ppm showed invading hyphae growing through epidermal cells into all cortical cells but not the vascular elements or pith (Plates 7 and 8). Transverse sections of infected hypocotyl tissues from plants treated with Demosan at 300 and 600 ppm showed hyphae concentrated in pockets formed in the epidermis and first layers of cortex cells. Hyphae were noted penetrating into the cortex. More invasion of hyphae was noted in plants treated at 300 than at 600 ppm (Plates 9, 10, 11 and 12). In the case of hypocotyl tissue treated with 900 and 1200 ppm, the fungus did not invade the epidermal cells and grew only on the surface of the hypocotyl (Plates 13, 14, 15, and 16). These results showed that Demosan or a compound related to it moved through all the cells of the hypocotyl and protected them from invasion by the test fungus only at the higher concentrations.

Evaluation of Demosan for Systemic Activity in Cotton Seedlings against R. solani

Two experiments were conducted in the laboratory using the glass dish technique to determine the systemic activity of Demosan in cotton seedlings against R. solani. In the first experiment, the treatments with Demosan with or without the fungus and noninfested check had approximately identical means of germinated seed after 10 days and had the same means of healthy seedlings after 3 weeks (Table 14). The

difference between these treatments and the infested check was highly significant at 1% level of probability (Table 14 and Plate 17).

In a similar test, Demosan used as a seed treatment at 9 oz/100 lb was compared with Panogen 15 at 3 oz and 2 oz/100 lb on both AD and MD cottonseed, and Demosan wettable powder incorporated in vermiculite at 600 ppm (Tables 15 and 16). The data from this experiment and later experiments was analyzed as a simple factorial experiment to evaluate the interaction. Demosan and MD cottonseed appeared to give better germination than Panogen 15 and AD seed. This difference was highly significant at 1% level of probability (Table 15). The number of healthy seedlings was recorded every 5 days beginning with the tenth day and continuing until the thirtieth day. The interaction /fungicide (Demosan and Panogen) x fungus (with and without fungus x seed (AD and MD)/ was highly significant after 10, 15, 20, 25, and 30 days. A comparison therefore was made between the individual means (Table 16). Demosan appeared to give better protection when incorporated in the growing medium than when used as seed treatment (Table 16).

These results showed that Demosan gave good control against R. solani in cotton seedlings when it was used at 600 ppm using the glass dish technique in the laboratory (Tables 14 and 16).

Using the same experimental design in the greenhouse, but using either nonsterile or sterile soil instead of vermiculite, opposite results were obtained. The percentage of healthy seedlings for the individual treatments was determined in an average for both soils. The interaction of fungicide x fungus x seed was highly significant at 1% level of probability. In these experiments, the seed treatment

with Demosan gave better and longer protection than when incorporated in soil at .32 gm/pot (Table 20). There was no significant difference between sterile and nonsterile soil when they were used for testing Demosan in the greenhouse. There was high significant difference at 1% level of probability between both fungicides (Demosan, Panogen) both kind of seed (AD, MD), and both infested and noninfested soil as an average of all treatments (Tables 17, 18, and 19). In both experiments Demosan, noninfested soil, and MD seed gave better germination and better protection than Panogen, infested soil, and AD seed (Tables 17, 18, 19). These results were similar to the results of the laboratory experiments.

A greenhouse experiment was designed to compare five rates (0, .16, .32, .64 and .96 gm/pot) of Demosan as a soil treatment for control of cotton soreshin with Demosan added to the soil at two dates: either 6 days before planting time or at planting time. There was a highly significant difference at 1% level of probability between all noninfested and all infested soil, and the noninfested soil gave better percentage of germination and healthy seedlings than the infested soil (Table 21). The highest rate of .96 gm/pot was phytotoxic and reduced percentage of germination, accordingly, it reduced the percentage of healthy seedlings. There was no significant difference between the dates of adding Demosan to the soil, and also the interaction (rates of Demosan x dates x fungus) was not significant. But the interaction (rates of Demosan x dates) was significant only at 5% level of probability only in the case of percentage of germination after 10 days and percentage of healthy seedlings after 20, 25, and

30 days (Table 22). Demosan gave the best protection against R. solani at .64 gm/pot and the difference between this rate and the nontreated check was significant only after 20, 25, and 30 days when it was applied to the soil at time of planting and the difference was not significant in the same periods when it was applied to the soil 6 days before planting (Table 22).

Comparison of Vitavax and Plantvax to Demosan for Systemic Activity in Cotton Seedlings against R. solani

The glass dish technique and clay pot technique was used for this evaluation. Vitavax, Plantvax, and Demosan also were compared to Panogen 15 as seed treatments on both AD and MD cottonseed. Three experiments were conducted, one in the laboratory and two in the greenhouse. The design of the first two experiments was exactly the same, and in the third experiment Vitavax and Plantvax were compared as soil treatments in the greenhouse at the rate of .15 gm/pot. These experiments were analyzed as simple factorial experiments in order to evaluate the interactions.

In the laboratory experiment the analysis showed significant differences at 1% level of probability between fungicides, and Demosan gave better percentage of germination and better percentage of healthy seedlings followed by Vitavax, Plantvax, and Panogen, respectively (Tables 23 and 24). There was no significant difference in germination between noninfested and infested treatments with R. solani, but the noninfested treatments gave a higher percentage of healthy seedlings than the infested treatments, and the difference was highly significant at 1% level of probability (Table 25). The MD seed germinated better than the AD seed and the difference was significant at

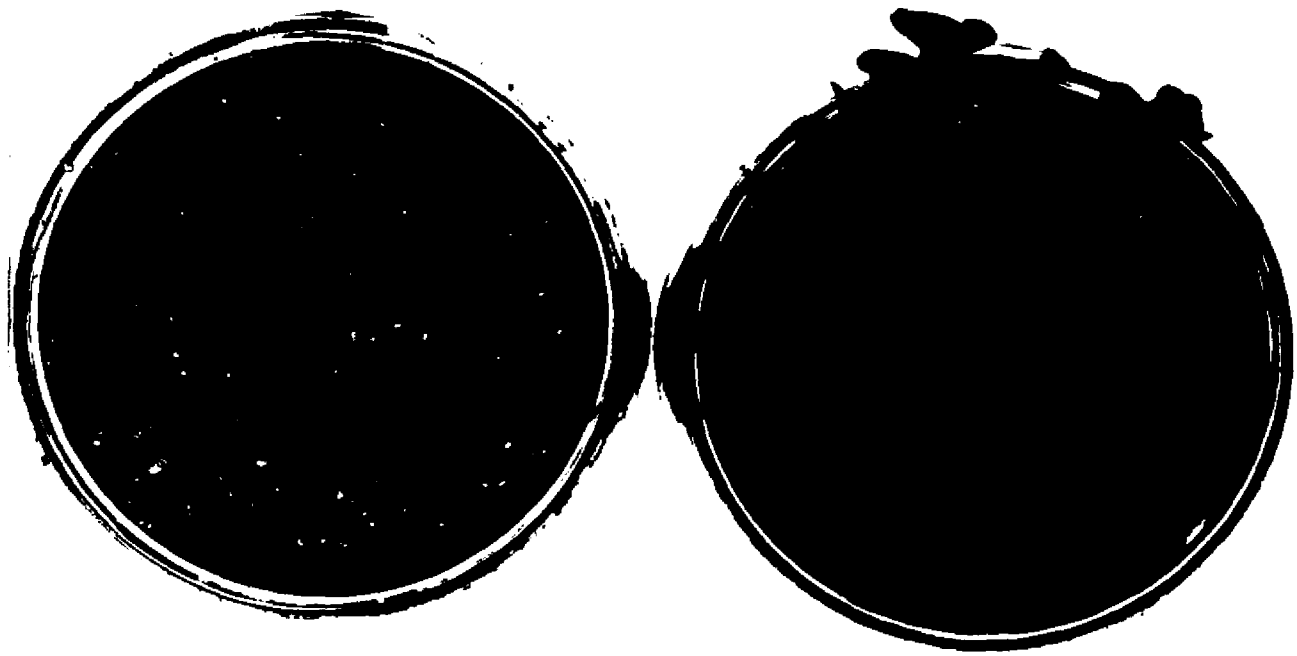

Plate 17. Comparison between nontreated check (at left) and 600 ppm of Demosan 75% wettable powder formulation (at right) 15 days after sowing using the glass dish technique. Both treatments were inoculated with R. solani.

Table 14. Mean (3 reps.) per cent of germinated seed and healthy seedlings from 60 seed in sterile vermiculite either nontreated or treated with Demosan at 600 ppm and either noninfested or infested with R. solani using the glass dish technique.

Treatments	Infested with R. solani	Mean per cent of germinated seed at 10 days after sowing	Mean per cent of healthy seedlings at 21 days after sowing
Check	without	83.33	83.33
Check	with	41.66	0.00
Demosan	without	83.33	81.66
Demosan	with	81.66	81.66
L.S.D. at 0.05		16.56	14.13
L.S.D. at 0.01		24.09	20.56

Table 15. Mean (4 reps.) per cent of germinated seed for both fungicides and both kind of seed as an average of all treatments of each of them after 10 days using the glass dish technique.

Fungicide		Kind of seed	
Demosan	Panogen	Acid-delinted	Machine-delinted
90.41	70.83	74.16	87.08
L.S.D. at 0.05 = 6.9324		L.S.D. at 0.05 = 6.9324	
L.S.D. at 0.01 = 9.5484		L.S.D. at 0.01 = 9.5484	

Table 16. Mean (4 reps.) per cent of healthy seedlings from 60 seed either nontreated or treated with Demosan or Panogen 15 at rates indicated in sterile vermiculite either noninfested or infested with R. solani using AD or MD seed and the glass dish technique.

Fungicide	Rate	R. solani	Seed	Mean per cent of healthy seedlings at days after sowing				
				10	15	20	25	30
1. Demosan	600 ppm	no	AD	91.66	91.66	91.66	91.66	91.66
2. Demosan	600 ppm	yes	AD	88.33	88.33	88.33	88.33	88.33
3. Demosan	9 oz/100#	no	MD	93.33	93.33	93.33	93.33	93.33
4. Demosan	9 oz/100#	yes	MD	20.00	1.66	0.00	0.00	0.00
5. Panogen 15	3 oz/100#	no	MD	83.33	83.33	83.33	83.33	83.33
6. Panogen 15	3 oz/100#	yes	MD	8.33	1.66	0.00	0.00	0.00
7. Panogen 15	2 oz/100#	no	AD	58.33	58.22	58.33	58.33	58.33
8. Panogen 15	2 oz/100#	yes	AD	3.33	0.00	0.00	0.00	0.00
L.S.D. at 0.05				12.5080	9.6672	9.3128	9.3128	9.3128
L.S.D. at 0.01				17.2280	13.3142	12.9538	12.9538	12.9538

Table 17. Mean (4 reps.) per cent of germinated seed for Demosan or Panogen 15, AD or MD seed, and noninfested or infested soil as an average of all treatments used after 10 days under greenhouse conditions using the clay pot technique.

Experiment I						Experiment II					
Fungicide		R. solani		Kind of seed		Fungicide		R. solani		Kind of seed	
Demosan	Panogen 15	No	Yes	AD	MD	Demosan	Panogen 15	No	Yes	AD	MD
81.66	48.12	77.08	52.70	58.74	71.03	72.91	42.70	64.58	51.03	38.95	76.66
L.S.D. at 0.05 = 6.97 0.01 = 9.29		L.S.D. at 0.05 = 6.97 0.01 = 9.29		L.S.D. at 0.05 = 6.97 0.01 = 9.29		L.S.D. at 0.05 = 7.01 0.01 = 9.21		L.S.D. at 0.05 = 7.01 0.01 = 9.21		L.S.D. at 0.05 = 7.01 0.01 = 9.21	

Table 18. Mean (4 reps.) per cent of healthy seedlings for Demosan or Panogen 15, AD or MD seed, and noninfested or infested soil as an average of all treatments used under greenhouse conditions using the clay pot technique. (First experiment.)

	Mean per cent of healthy seedlings at days after sowing				
	10	15	20	25	30
Fungicide					
Demosan	68.12	59.58	53.95	49.37	45.62
Panogen 15	35.41	34.37	31.87	31.66	30.83
L.S.D. at 0.05	5.61	5.67	6.23	6.01	5.73
L.S.D. at 0.01	7.47	7.55	8.31	8.01	7.63
Kind of seed					
AD	46.66	41.87	37.70	34.99	32.28
MD	56.87	52.08	48.12	46.03	44.16
L.S.D. at 0.05	5.61	5.67	6.23	6.01	5.73
L.S.D. at 0.01	7.47	7.55	8.31	8.01	7.63
R. solani					
No	77.08	77.08	77.08	77.08	77.08
Yes	26.45	16.87	11.24	6.45	1.87
L.S.D. at 0.05	5.61	5.67	6.23	6.01	5.73
L.S.D. at 0.01	7.47	7.55	8.31	8.01	7.63

Table 19. Mean (4 reps.) per cent of healthy seedlings for Demosan or Panogen 15, AD or MD seed, and noninfested or infested soil as an average of all treatments used under greenhouse conditions using the clay pot technique. (Second experiment.)

	Mean per cent of healthy seedlings at days after sowing				
	10	15	20	25	30
Fungicide					
Demosan	67.49	59.16	54.37	48.33	45.20
Panogen 15	37.91	32.08	28.74	26.45	26.03
L.S.D. at 0.05	6.01	6.99	7.07	6.51	6.25
L.S.D. at 0.01	8.01	9.32	9.43	8.68	8.33
Kind of seed					
AD	34.16	28.33	27.49	26.45	24.58
MD	71.24	62.91	55.62	48.33	46.66
L.S.D. at 0.05	6.01	6.99	7.07	6.51	6.25
L.S.D. at 0.01	8.01	9.32	9.43	8.68	8.33
R. solani					
No	64.58	64.58	64.58	64.58	64.58
Yes	40.82	26.66	18.53	10.20	6.66
L.S.D. at 0.05	6.01	6.99	7.07	6.51	6.25
L.S.D. at 0.01	8.01	9.32	9.43	8.68	8.33

Table 20. Mean (4 reps.) per cent of healthy seedlings for Demosan or Panogen 15, AD or MD seed, and non-infested or infested soil as an average for both sterile and nonsterile soil under greenhouse conditions using the clay pot technique.

Fungicide	Rate	R. solani	Seed	Experiment I: Mean per cent of healthy seedlings at days after sowing					Experiment II: Mean per cent of healthy seedlings at days after sowing				
				10	15	20	25	30	10	15	20	25	30
1. Demosan	.32 gm/pot	no	AD	83.32	83.32	83.32	83.32	83.32	64.99	64.99	64.99	64.99	64.99
2. Demosan	.32 gm/pot	yes	AD	43.33	25.83	19.16	9.16	00.83	32.49	12.39	9.99	7.49	1.66
3. Demosan	9 oz/100#	no	MD	91.66	91.66	91.66	91.66	91.66	90.83	90.83	90.83	90.83	90.83
4. Demosan	9 oz/100#	yes	MD	54.16	37.49	21.66	13.33	6.66	81.66	68.33	41.66	19.99	13.33
5. Panogen 15	3 oz/100#	no	MD	78.33	78.33	78.33	78.33	78.33	72.49	72.49	72.49	72.49	72.49
6. Panogen 15	3 oz/100#	yes	MD	3.33	00.83	00.83	00.83	00.00	39.99	19.99	7.49	00.00	00.00
7. Panogen 15	2 oz/100#	no	AD	54.99	54.99	54.99	54.99	54.99	29.99	29.99	29.99	29.99	29.99
8. Panogen 15	2 oz/100#	yes	AD	4.99	3.33	3.33	2.49	00.00	9.16	5.83	5.00	3.33	1.66
L.S.D. at 0.05				11.10	11.40	12.41	11.84	11.50	12.06	13.93	14.23	13.04	12.51
L.S.D. at 0.01				14.79	NS	NS	15.78	15.32	15.98	18.61	18.97	17.39	16.66

Table 21. Mean (4 reps.) per cent of germinated seed and healthy seedlings from soil either noninfested or infested with R. solani, and either nontreated or treated with Demosan applied either 6 days before planting or at planting as an average of all treatments (0, .16, .32, .64, and .96 gm/pot) under greenhouse conditions using the clay pot technique.

R. solani	Mean per cent of germinated seed after 10 days	Mean per cent of healthy seedlings at days after sowing				
		10	15	20	25	30
No	57.83	57.83	57.83	57.83	57.83	57.83
Yes	44.99	36.49	17.99	10.16	4.83	3.49
L.S.D. at 0.05	6.74	7.28	6.84	5.94	5.52	5.38
L.S.D. at 0.01	8.96	9.68	8.43	7.90	7.34	7.34

Table 22. Mean (4 reps.) per cent of germinated seed and healthy seedlings from soil either nontreated or treated with Demosan applied at 5 rates either 6 days before planting or at planting as an average for both noninfested and infested soil with R. solani.

Rate of Demosan in gm/pot	Mean per cent of germinated seed after 10 days in Demosan treatment at		Mean per cent of healthy seedlings at days after sowing									
			10		15		20		25		30	
	Planting	6 days before plant.	At plant.	6 days before plant.	At plant.	6 days before plant.	At plant.	6 days before plant.	At plant.	6 days before plant.	At plant.	6 days before plant.
0.00	37.49	61.66	30.83	49.16	27.49	42.49	25.83	39.99	24.16	38.33	23.33	38.33
0.16	54.16	45.82	44.99	43.32	33.33	33.33	29.16	31.66	26.66	29.16	26.66	28.33
0.32	58.33	46.66	53.32	45.82	44.16	34.99	39.99	29.99	35.83	25.83	35.83	24.99
0.64	59.16	61.66	57.49	59.99	46.66	47.49	43.33	39.99	39.99	37.49	37.49	36.66
0.96	53.32	35.83	50.83	35.83	41.66	27.49	36.66	23.33	33.32	22.49	32.49	22.49
L.S.D. at 0.05	16.66		NS		NS		13.30		12.40		12.08	
L.S.D. at 0.01	22.15		NS		NS		NS		NS		NS	

5% level of probability (Table 23), but the AD seed gave better percentage of healthy seedlings than MD seed only after 20, 25, and 30 days and the difference was significant only at the 5% level of probability (Table 25). The interaction (fungus x fungicide x seed) was highly significant at 1% level of probability in the analysis of percentage of healthy seedlings after 10, 15, 20, 25, and 30 days, therefore, the comparison was made between the individual means (Table 26). All seedlings in noninfested treatment remained healthy until the end of the experiment and Demosan gave less protection in infested soil than when incorporated in vermiculite at 600 ppm (Tables 26, 29, and 32). As a seed treatment Vitavax at 4 oz/100 lb gave better protection than Plantvax, Demosan, and Panogen. These results showed Demosan, Vitavax, and Plantvax as seed treatments under laboratory conditions, gave protection to cotton seedlings for about 10 days for Demosan, about 3 weeks for Vitavax, and about 2 weeks for Plantvax.

Using the same experimental design, but using either nonsterile or sterile soil instead of vermiculite, opposite results were obtained (Table 29). The interaction (soil x fungus x fungicide x seed) was significant in the analysis of percentage of germinated seed and in the analysis of percentage of healthy seedlings only after 10, 15, and 25 days. Individual means showed seed treatment with Demosan at 9 oz/100 lb gave better and longer protection than when incorporated in soil at .32 gm/pot (Table 29). Also, Demosan gave better protection in sterile soil than in nonsterile soil, but Vitavax gave better protection in nonsterile soil than in sterile soil (Table 29). There was a significant difference between fungicides, between noninfested and infested treatments, between AD and MD seed, and between nonsterile

and sterile soil at 1% level of probability (Tables 27, 28, 30, and 31). Demosan, noninfested soil, MD seed, and nonsterilized soil gave better percentage of germination and better percentage of healthy seedlings than Vitavax, Plantvax, Panogen, AD seed, infested soil, and sterilized soil as an average for all treatments of each of them (Tables 27, 28, 30, and 31). In the second experiment in the greenhouse the interaction (soil x fungus x fungicides x seed) was not significant, but the interaction (fungus x fungicides x seed) was significant so the comparison between results was made (Table 32). Demosan gave better protection when used as seed treatment at 9 oz/100 lb than when it was used at .32 gm/pot as soil treatment and Vitavax and Plantvax gave better protection when they were used as soil treatment at .15 gm/pot than when used as seed treatment at 4 oz/100 lb (Table 32).

It was observed that Demosan appeared to stimulate germination. Seed treated with Demosan tended to germinate and emerge earlier than nontreated seed. Vitavax tended to delay germination about 2 to 3 days while Plantvax tended to delay germination about 3 to 4 days.

Bioassay of Demosan-treated Cotton Seedlings

The results of the experiments designed to determine if fungicidal activity could be detected in Demosan-treated cotton seedlings are summarized (Table 33, and Plates 18, 19, and 20). These results indicated that there was an effect of the various concentrations of Demosan-treated-cotton-seedling extract on the growth of R. solani on PDA. There was a decrease in radial growth with an increase of the fungicide-treated-cotton-seedling extracts using the 75% wettable powder formulation of Demosan (Plates 18, 19, and 20). Duncan's

Table 23. Mean (3 reps.) per cent of germinated seed for Demosan, Vitavax, Plantvax, Panogen, and AD and MD seed as an average for all treatments of each of them after 10 days using the glass dish technique.

Fungicide				Kind of seed	
Demosan	Vitavax	Plantvax	Panogen	AD	MD
90.41	77.91	73.33	70.83	73.95	82.29
L.S.D. at 0.05 = 9.29 L.S.D. at 0.01 = 12.55				L.S.D. at 0.05 = 6.56	

Table 24. Mean (3 reps.) per cent of healthy seedlings for Demosan, Vitavax, and Plantvax as an average for all treatments of each of them using the glass dish technique.

	Mean per cent of healthy seedlings at days after sowing				
Fungicide	10	15	20	25	30
Demosan	73.33	68.75	68.33	68.33	68.33
Vitavax	73.33	62.50	50.83	48.75	45.00
Plantvax	55.83	46.25	40.41	39.16	39.16
Panogen	38.33	35.83	35.41	35.41	35.41
L.S.D. at 0.05	9.71	9.75	8.32	8.32	7.85
L.S.D. at 0.01	13.13	13.18	11.24	11.24	10.61

Table 25. Mean (3 reps.) per cent healthy seedlings for noninfested or infested treatments with R. solani and for AD and MD seed as an average of all treatments of each of them using the glass dish technique at days indicated after sowing.

	Mean per cent of healthy seedlings at days after sowing						Mean per cent of healthy seedlings at days after sowing				
R. solani	10	15	20	25	30	Kind of seed	10	15	20	25	30
No	79.58	79.58	79.58	79.58	79.58	AD	61.45	56.66	51.66	51.25	50.62
Yes	40.83	27.08	17.70	16.25	14.37	MD	58.95	50.00	45.62	44.58	43.33
L.S.D. at						L.S.D. at					
0.05	6.86	6.90	5.87	5.87	5.55	.0.05	NS	NS	5.87	5.87	5.55
0.01	8.18	8.63	7.94	7.94	7.50	0.01	NS	NS	NS	NS	NS

Table 26. Mean (3 reps.) per cent of healthy seedlings from 60 seed either nontreated or treated with Demosan or Vitavax or Plantvax or Panogen 15 at rates indicated in sterile vermiculite either noninfested or infested with R. solani using AD or MD seed and the glass dish technique.

Fungicide	Rate	R. solani	Seed	Mean per cent of healthy seedlings at days after sowing 10	15	20	25	30
1. Dempsan	600 ppm	no	AD	91.66	91.66	91.66	91.66	91.66
2. Demosan	600 ppm	yes	AD	88.33	88.33	88.66	88.33	88.33
3. Demosan	9 oz/100#	no	MD	93.33	93.33	93.33	93.33	93.33
4. Demosan	9 oz/100#	yes	MD	20.00	1.66	00.00	00.00	00.00
5. Vitavax	4 oz/100#	no	MD	76.66	76.66	76.66	76.66	76.66
6. Vitavax	4 oz/100#	yes	MD	58.33	40.00	23.33	18.33	8.33
7. Vitavax	4 oz/100#	no	AD	83.33	83.33	83.33	83.33	83.33
8. Vitatax	4 oz/100#	yes	AD	75.00	50.00	20.00	16.66	11.66
9. Plantvax	4 oz/100#	no	MD	80.00	80.00	80.00	80.00	80.00
10. Plantvax	4 oz/100#	yes	MD	51.66	23.33	8.33	5.00	5.00
11. Plantvax	4 oz/100#	no	AD	70.00	70.00	70.00	70.00	70.00
12. Plantvax	4 oz/100#	yes	AD	21.55	11.66	3.33	1.66	1.66
13. Panogen 15	3 oz/100#	no	MD	83.33	83.33	83.33	83.33	83.33
14. Panogen 15	3 oz/100#	yes	MD	8.33	1.66	00.00	00.00	00.00
15. Panogen 15	2 0z/100#	no	AD	58.33	58.33	58.33	58.33	58.33
16. Panogen 15	2 oz/100#	yes	AD	3.33	00.00	00.00	00.00	00.00
L.S.D. at 0.05				19.45	16.88	16.66	16.66	15.73
L.S.D. at 0.01				26.28	22.82	22.52	22.52	21.26

Table 27. Mean (3 reps.) per cent of germinated seed for Demosan, Vitavax, Plantvax, Panogen, AD and MD seed, and noninfested and infested treatments with R. solani as an average of all treatments of each of them in sterile and nonsterile soil under greenhouse conditions using the clay pot technique after 10 days.

Fungicide				R. solani		Seed	
Demosan	Vitavax	Plantvax	Panogen	No	Yes	AD	MD
81.66	68.64	61.03	48.12	73.27	56.45	60.77	68.95
L.S.D. at .0.05 = 6.93 0.01 = 9.20				L.S.D. at 0.05 = 5.03 0.01 = 6.38		L.S.D. at 0.05 = 5.03 0.01 = 6.38	

Table 28. Mean (4 reps.) per cent of healthy seedlings for Demosan, Vitavax, Plantvax, Panogen, infested or noninfested treatments with R. solani, AD or MD seed, and for sterile or nonsterile soil as an average for all treatments of each of them under greenhouse conditions using the clay pot technique.

	Mean per cent of healthy seedlings at days after sowing				
	10	15	20	25	30
Fungicide					
Demosan	67.91	59.37	53.74	49.37	45.41
Vitavax	64.78	58.74	50.62	45.41	38.33
Plantvax	44.37	40.83	39.16	37.91	37.91
Panogen 15	35.41	34.37	34.37	34.16	33.33
L.S.D. at 0.05	5.72	5.88	5.44	4.57	4.96
L.S.D. at 0.01	7.50	7.76	7.23	6.07	6.48
R. solani					
No	74.05	74.05	74.05	74.05	74.05
Yes	32.18	22.60	14.89	9.37	3.43
L.S.D. at 0.05	4.03	4.15	3.84	2.83	3.52
L.S.D. at 0.01	5.36	5.52	5.10	3.76	4.68
Kind of Seed					
AD	49.99	44.68	41.24	38.74	35.51
MD	56.24	51.97	47.70	44.68	41.97
L.S.D. at 0.05	4.03	4.15	3.84	2.83	3.52
L.S.D. at 0.01	5.36	5.52	5.10	3.76	4.68
Kind of Soil					
Nonsterile	54.99	51.55	48.43	45.41	41.87
Sterile	51.24	45.10	40.51	38.01	35.62
L.S.D. at 0.05	4.03	4.15	3.84	2.83	3.52
L.S.D. at 0.01	-	5.52	5.10	3.76	4.68

Table 29. Mean (4 reps.) per cent of germinated seed and healthy seedlings from 60 seed either nontreated or treated with Demosan or Vitavax or Plantvax or Panogen 15 at rates indicated in sterile (S) or nonsterile (NS) soil either noninfested or infested with R. solani using AD or MD seed under greenhouse conditions using the clay pot technique.

Fungicide	Rate	R. solani	Seed	Mean per cent of germinated seed after 10 days		Mean per cent of healthy seedlings at days after sowing									
						10		15		20*		25		30*	
				S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
1. Demosan	.32 gm/pot	no	AD	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33	83.33
2. Demosan	.32 gm/pot	yes	AD	73.33	66.66	54.87	31.66	33.33	18.33	19.99	16.66	9.95	8.32	1.66	0.00
3. Demosan	9 oz/100#	no	MD	91.66	91.66	91.66	91.66	91.66	91.66	91.66	91.66	91.66	91.66	91.66	91.66
4. Demosan	9 oz/100#	yes	MD	78.33	84.99	61.66	44.99	41.66	31.66	21.66	21.66	14.99	11.66	6.66	4.99
5. Vitavax	4 oz/100#	no	MD	64.99	74.99	64.99	74.99	64.99	74.99	64.99	74.99	64.99	74.99	64.99	74.99
6. Vitavax	4 oz/100#	yes	MD	63.33	81.66	54.99	76.66	39.99	69.99	23.32	54.99	18.33	29.99	8.33	4.99
7. Vitavax	4 oz/100#	no	AD	71.66	76.66	71.66	76.66	71.66	76.66	71.66	76.66	71.66	76.66	71.66	76.66
8. Vitavax	4 oz/100#	yes	AD	63.32	64.99	54.99	43.33	36.66	34.99	19.99	19.99	8.32	18.33	1.66	3.33
9. Plantvax	4 oz/100#	no	MD	54.99	89.99	54.99	89.99	54.99	89.99	54.99	89.99	54.99	89.99	54.99	89.99
10. Plantvax	4 oz/100#	yes	MD	59.16	48.33	16.66	13.30	9.99	11.66	4.99	11.66	3.33	9.99	3.33	3.33
11. Plantvax	4 oz/100#	no	AD	68.33	66.66	68.33	66.66	68.33	66.66	68.33	66.66	68.33	66.66	68.33	66.66
12. Plantvax	4 oz/100#	yes	AD	44.99	58.33	18.33	26.66	8.33	16.66	6.66	9.99	1.66	8.33	0.00	1.66
13. Panogen 15	3 oz/100#	no	MD	71.66	84.99	71.66	84.99	71.66	84.99	71.66	84.99	71.66	84.99	71.66	84.99
14. Panogen 15	3 oz/100#	yes	MD	44.99	19.99	3.33	3.33	0.00	1.66	0.00	1.66	0.00	1.66	0.00	0.00
15. Panogen 15	2 oz/100#	no	AD	41.66	68.33	41.66	68.33	41.66	68.33	41.66	68.33	41.66	68.33	41.66	68.33
16. Panogen 15	2 oz/100#	yes	AD	38.33	15.00	6.66	3.33	3.33	3.33	3.33	3.33	3.33	1.66	0.00	0.00
L.S.D. at 0.05				19.64		16.21		16.65		-		12.94		-	
L.S.D. at 0.01				-		21.53		-		-		-		-	

*There is no significant difference.

Table 30. Mean (4 reps.) per cent of germinated seed for Demosan, Vitavax, Plantvax, Panogen 15, AD and MD seed, and noninfested and infested treatments with R. solani as an average of each of them in sterile and nonsterile soil under greenhouse conditions using the clay pot technique after 10 days.

	Fungicide					R. solani		Seed	
	Vitavax		Plantvax						
Demosan	Seed treat.	Soil treat.	Seed treat.	Soil treat.	Panogen	No	Yes	AD	MD
72.91	43.34	65.83	39.58	60.62	44.58	57.29	51.66	40.97	67.48
L.S.D. at 0.05			6.72			3.86		3.86	
L.S.D. at 0.01			8.81			5.06		5.06	

Table 31. Mean (4 reps.) per cent of healthy seedlings for Demosan, Vitavax (seed or soil treatments), Plantvax (seed or soil treatment), Panogen, infested or noninfested treatments with R. solani, AD or MD seed, and for sterile or nonsterile soil as an average for all treatments of each of them under greenhouse conditions using the clay pot technique.

	Mean per cent of healthy seedlings at days after sowing				
	10	15	20	25	30
Fungicide					
Demosan	66.03	57.08	51.24	45.83	42.70
Vitavax	42.71	40.42	37.50	32.09	27.92
Plantvax	37.49	35.62	32.49	28.74	26.45
Panogen 15	39.37	33.12	29.16	26.45	26.03
Vitavax (Soil treat.)	61.45	56.45	51.24	43.95	41.66
Plantvax (Soil treat.)	56.24	49.99	42.91	37.28	35.62
L.S.D. at 0.05	6.61	7.27	7.60	7.33	6.99
L.S.D. at 0.01	8.66	9.53	9.97	9.61	9.17
R. solani					
No	57.15	57.15	57.15	57.15	57.15
Yes	43.95	33.88	24.92	14.78	10.13
L.S.D. at 0.05	3.82	4.19	4.39	4.23	4.03
L.S.D. at 0.01	5.01	5.49	5.75	5.55	5.29
Kind of Seed					
AD	37.70	33.95	31.18	27.98	26.45
MD	63.39	56.94	50.34	43.46	40.34
L.S.D. at 0.05	3.82	4.19	4.39	4.23	4.03
L.S.D. at 0.01	5.01	5.49	5.75	5.55	5.29
Kind of Soil					
Nonsterile	52.29	47.77	43.47	38.54	36.59
Sterile	48.81	43.12	38.05	32.91	30.20
L.S.D. at 0.05	NS	4.19	4.39	4.23	4.03
L.S.D. at 0.01	NS	NS	NS	NS	5.29

Table 32. Mean (4 reps.) per cent of germinated seed and healthy seedlings from 120 seeds nontreated or treated with Demosan or with Vitavax (seed or soil treatment) or Plantvax (seed or soil treatment) or Panogen 15 at rates indicated in soil either infested or noninfested with R. solani using AD or MD seed as an average for all soils (sterile and nonsterile) under greenhouse conditions using the clay pot technique.

Fungicide	Rate	R. solani	Seed	Mean per cent of germinated seed after 10 days	Mean per cent of healthy seedlings after sowing				
					10	15	20	25	30
1. Demosan	.32 gm/pot	no	AD	64.99	64.99	64.99	64.99	64.99	64.99
2. Demosan	.32 gm/pot	yes	AD	46.66	32.49	12.49	9.99	7.49	1.66
3. Demosan	9 oz/100#	no	MD	90.83	90.83	90.83	90.83	90.83	90.83
4. Demosan	9 oz/100#	yes	MD	89.16	75.82	59.99	41.66	19.99	13.33
5. Vitavax	4 oz/100#	no	MD	51.66	51.66	51.66	51.66	51.66	51.66
6. Vitavax	4 oz/100#	yes	MD	64.99	62.49	57.49	48.33	31.66	17.49
7. Vitavax	4 oz/100#	no	AD	30.87	30.87	30.87	30.87	30.87	30.87
8. Vitavax	4 oz/100#	yes	AD	25.82	25.82	21.66	19.16	14.16	11.66
9. Plantvax	4 oz/100#	no	MD	46.66	46.66	46.66	46.66	46.66	46.66
10. Plantvax	4 oz/100#	yes	MD	57.49	49.99	43.33	33.33	22.49	17.49
11. Plantvax	4 oz/100#	no	AD	31.66	31.66	31.66	31.66	31.66	31.66
12. Plantvax	4 oz/100#	yes	AD	22.49	21.66	20.83	18.33	14.16	9.99
13. Panogen 15	3 oz/100#	no	MD	72.49	72.49	72.49	72.49	72.49	72.49
14. Panogen 15	3 oz/100#	yes	MD	54.16	39.99	19.99	7.49	0.00	0.00
15. Panogen 15	3 oz/100#	no	AD	34.16	34.16	34.16	34.16	34.16	34.16
16. Panogen 15	3 oz/100#	yes	AD	17.49	12.49	9.16	8.33	6.66	4.99

Continued

Table 32. Continued.

Fungicide	Rate	R. solani	Seed	Mean per cent of germinated seed after 10 days	Mean per cent of healthy seedlings after sowing				
					10	15	20	25	30
17. Vitavax	.15 gm/pot	no	MD	76.66	76.66	76.66	76.66	76.66	76.66
18. Vitavax	.15 gm/pot	yes	MD	74.16	67.49	57.49	44.99	28.32	22.49
19. Vitavax	.15 gm/pot	no	AD	64.16	64.16	64.16	64.16	64.16	64.16
20. Vitavax	.15 gm/pot	yes	AD	48.33	37.49	27.49	19.16	6.66	3.33
21. Plantvax	.15 gm/pot	no	MD	75.83	75.83	75.83	75.83	75.83	75.83
22. Plantvax	.15 gm/pot	yes	MD	61.66	52.49	34.16	22.49	12.49	6.66
23. Plantvax	.15 gm/pot	no	AD	47.49	47.49	47.49	47.49	47.49	47.49
24. Plantvax	.15 gm/pot	yes	AD	57.49	49.16	42.49	25.83	13.33	12.49
L. S. D. at 0.05				13.44	13.21	14.56	15.22	14.68	13.99
L. S. D. at 0.01				NS	17.32	19.09	19.96	NS	NS

Multiple Range Test for significance was applied to all the data (Table 33). The statistical analysis showed significant differences between the different concentrations and the nontreated checks. It appeared from these experiments that the Demosan-treated-cotton-seedlings-extract had direct effect against R. solani in vitro.

In Vitro Studies

In vitro studies were performed to determine if Demosan had direct action on the fungus. There was a decrease in radial growth with an increase in fungicide concentration using the 75% formulation (Plates 21 and 22). Duncan's Multiple Range Test for significance was applied to test the significance between the various concentrations. The analyses show that there was significant differences between the different concentrations and the nontreated checks, and Demosan prevented the fungus growth completely at 150 ppm (Table 34).

Determination of the Effect of Demosan on Reducing Sugars in Cotton Seedlings

The results of the experiment designed to determine if Demosan had any effect on the quantity of reducing sugars in cotton seedlings and if any changes in the amount could be correlated to susceptibility to R. solani are summarized in Tables 35 and 36. Duncan's Multiple Range Test for significance was applied to test the data (Table 36). These results indicated that there was a decrease in the amount of reducing sugars in cotton seedlings with an increase in fungicide concentration using the 75% formulation. The statistical analyses indicated that the differences in the amount of reducing sugars in

Table 33. Mean (4 reps.) radial growth, in cm., of R. solani after 4 days at 26 C on PDA without or with hypocotyl-tissue-extracts from Demosan-treated cotton seedlings after 30 hours exposure (Expt. II) and 7 days after sowing (Expt. I).

Rate of Demosan in ppm	Mean[2/] radial growth in cm for Expt. II	Expt. I
0[1/]	9.0 a	9.0 a
0	9.0 a	9.0 a
100	3.2 b	8.8 b
200	2.4 c	8.4 c
300	2.2 d	8.2 d
600	1.7 e	7.7 e
800	-	7.6 f
900	1.5 f	7.4 g
1200	1.3 g	4.9 h

[1/] 0 = Without tissue extract.

[2/] Means followed by the same letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

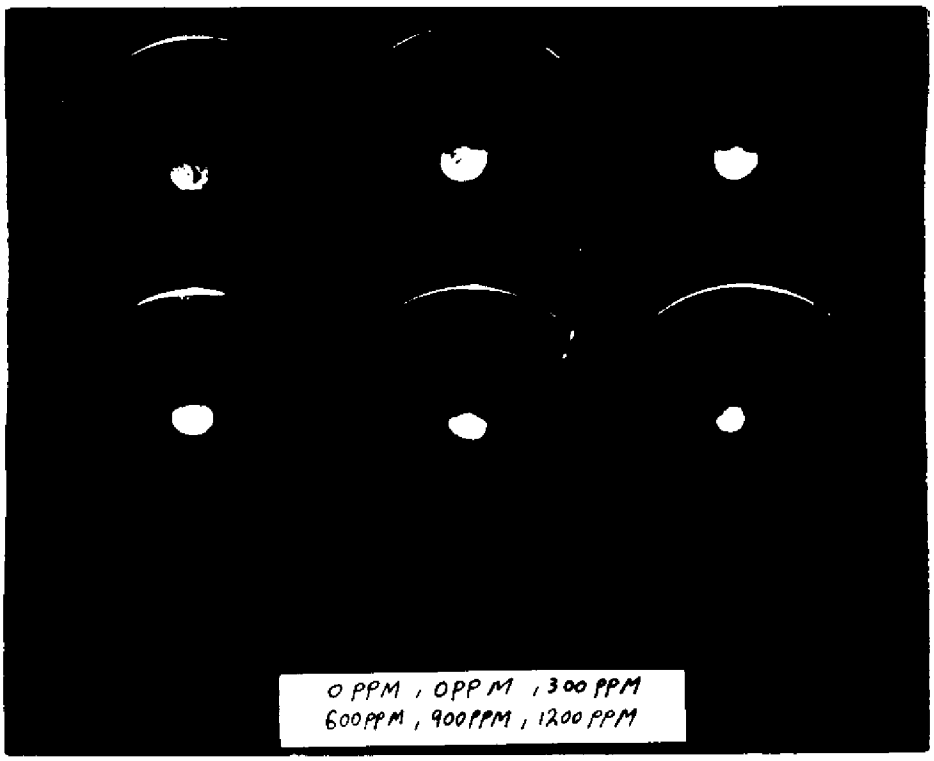

Plate 18. Culture plates showing radial growth of R. solani on PDA either without or with various concentrations of Demosan-treated cotton seedlings (7-day-old) 15 hours after seeding. (Left to right, top to bottom): 0 ppm without tissue extract, and 0, 300, 600, 900, 1200 ppm with tissue extract.

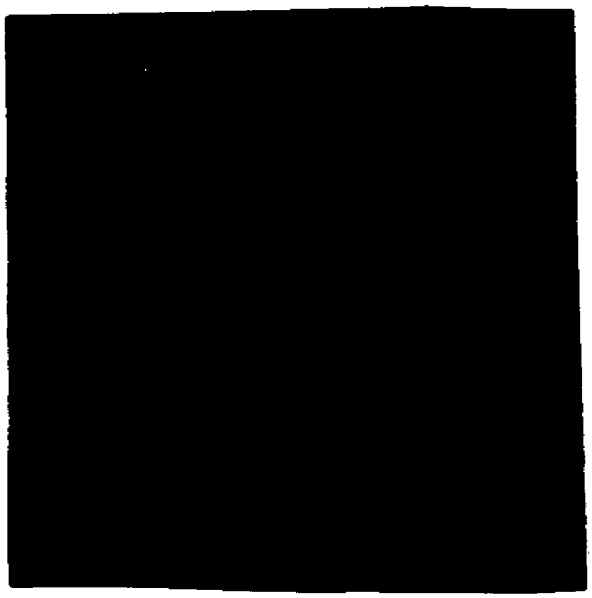

Plate 19. Culture plates showing radial growth of R. solani on PDA either without or with various concentrations of Demosan-treated cotton seedlings (7-days old) 5 days after seeding. (Left to right, top to bottom): 0 ppm without tissue extract, and 0, 100, 200, 300, 600, 800, 900, 1200 ppm with tissue extract.

Plate 20. Cultural plates showing radial growth of R. solani on PDA either without or with various concentrations of Demosan-treated cotton seedlings (for 30 hours) 4 days after seeding. (Left to right, top to bottom): 0 ppm without tissue extract, and 0, 100, 200, 300, 600, 900, 900, 1200 ppm with tissue extract.

Table 34. Mean radial growth, in cm, of R. solani on PDA either with or without the 75% wettable powder formulation of Demosan 4 days after seeding at 26 C at rates indicated.

Rate in ppm	Mean radial growth[1/] In the first run	In the second run
0	9.0 a	9.0 a
15	2.6 b	2.5 b
25	2.1 c	2.1 c
50	1.9 cd	1.9 d
75	1.5 e	1.5 e
100	1.4 ef	1.3 f
125	1.2 efg	1.2 g
150	.	0.0 h

[1/] Means followed by same letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

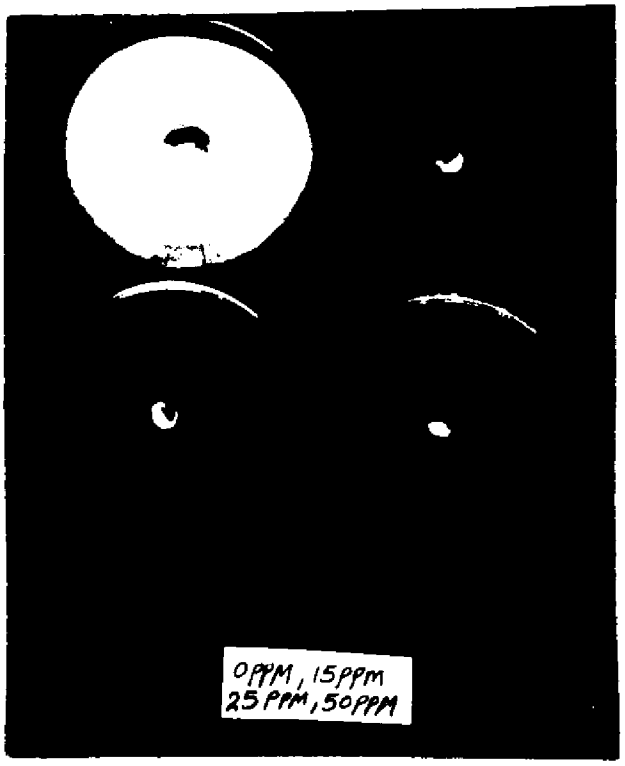

Plate 21. Culture plates showing radial growth of R. solani on PDA containing different rates of the 75% wettable powder formulation of Demosan. (Left to right, top to bottom): 0, 15, 25, and 50 ppm, after 4 days at 26 C.

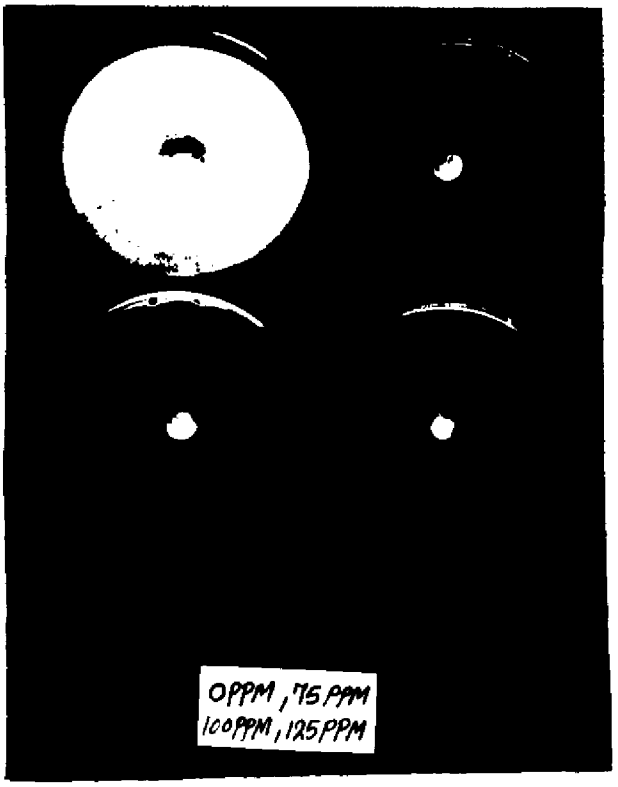

Plate 22. Culture plates showing radial growth of R. solani on PDA containing different rates of the 75% wettable powder formulation of Demosan. (Left to right, top to bottom): 0, 75, 100, 125 ppm, after 4 days at 26 C.

Table 35. Mean of the amount of reducing sugar in 0.25 gm of dry tissue, in ugm, from the average of two runs of the experiment each with 3 replications after 7 days.

Rate of Demosan in ppm	R. solani	Mean of the amount of reducing sugar in ugm[1/]
0	no	1706.03
0	yes	1694.78
100	yes	1719.16
300	yes	1700.81
600	yes	1684.35
900	yes	1678.11
1200	yes	1708.03

[1/] There is no significant difference between treatments.

Table 36. Mean of the amount of reducing sugar in 0.25 gm of dry tissue, in ugm, from the average of two runs of the experiment each with 3 replications after 15 days.

Rate of Demosan in ppm	R. solani	Mean of the amount of reducing sugar in ugm[1/]
0	no	1444.78 a
0	yes	1743.95 bcde
100	yes	1418.73 cdef
300	yes	1264.56 defg
600	yes	1221.43 efg
900	yes	1209.36 fg
1200	yes	1135.40 g

[1/] Means followed by same letter are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

cotton seedlings using different concentrations of Demosan was not significant after 7 days from sowing, but the difference was significant after 15 days from sowing (Tables 35 and 36). The highest amount of reducing sugars was in the nontreated-inoculated check followed by the nontreated-noninoculated check. These results indicate a relationship between the susceptibility to R. solani and Demosan.

DISCUSSION

There were no published works on the use of systemic fungicides for the protection of cotton seedlings against infection by R. solani at the beginning of this research. Both laboratory and greenhouse techniques were developed to test different fungicides for their systemic activity in cotton seedling hypocotyls against the fungus. Four techniques were developed: the glass dish and flask techniques for use in the laboratory; and the flat and clay pot techniques for use in the greenhouse.

Results from the comparison of variations within these techniques indicated that the reason for high percentage of healthy seedlings in the nontreated-infested pots and dishes in variation 3 was because cotton seedlings were exposed to fungus invasion 1 week less than the other two variations. The nontreated-infested containers in variation 1 both in glass dish and clay pot techniques gave the lowest percentage of healthy seedlings, while the highest percentage of germinated seed and healthy seedlings was in the treated-noninfested containers. The methods also were found to be suited for observing any growth stimulation or phytotoxicity due to the fungicide. Demosan, for instance, showed both stimulation to seedling growth and increased cottonseed germination at certain levels.

Results obtained in the evaluation of the greenhouse flat technique were not satisfactory because they had a low percentage of germination, and consequently gave a low percentage of healthy

seedlings. Another reason was that at the end of the experiments, several seedlings were found growing under the bottom of the top flat for several replicates.

It was found during the comparison of variations in the flask technique that the incorporation of vermiculite in flasks allowed for a better evaluation of disease control than the other variations. There were probably two reasons for this: First, the vermiculite offered support of the seedlings after the roots had grown into the medium; and second, the vermiculite might have facilitated a more equitable distribution of the fungicide in the flask, as well as preventing it from settling to the bottom of the flask, thus making it readily available to the seedling roots. Little or no differences were noted between the use of distilled water or Hoagland's solution as a medium for the suspension of the fungicide. The flask technique, therefore, using distilled water and vermiculite was used in the laboratory for the determination of the systemic chemotherapeutic activity of Demosan against R. solani on cotton seedlings.

Two experiments were conducted in the laboratory to test the systemic activity of Demosan using the glass dish technique. Demosan as a seed treatment at 9 oz/100 lb was compared with: Panogen 15 at 2 oz/100 lb on both AD and MD cottonseed; and Demosan wettable powder (WP) incorporated in vermiculite at 600 ppm using the glass dish technique. Demosan gave better protection, as shown by an increase in the percentage of healthy seedlings, when incorporated in vermiculite at 600 ppm than when used as seed treatment at 9 oz/100 lb. This difference was highly significant. Thus, the glass dish

technique proved to be satisfactory for the evaluation of systemic fungicides in cotton seedlings.

Using the same experimental design in the greenhouse, but using either nonsterile or sterile soil instead of vermiculite similar results were obtained except in the seed treatment studies. In these experiments Demosan gave better and longer protection than when incorporated in soil at .32 gm/pot and there was no significant difference between sterile and nonsterile soil. The clay pot technique was shown then to be suitable for evaluating Demosan as seed treatments and detecting systemic activity.

In the greenhouse there was no significant difference between dates of adding five rates of Demosan to the soil. Phytotoxicity occurred, however, on seedlings in soil treated with .96 gm/pot Demosan and this reduced both percentage of germination and percentage of healthy seedlings. It also caused some stunting of the plants. This phytotoxicity weakened the seedlings, apparently making them more susceptible to fungus invasion. The greenhouse technique not only was successful in evaluating systemic activity of Demosan, but also in detecting phytotoxicity.

Results of the laboratory experiments showed a highly significant difference between fungicides. Demosan gave the best percentage of germination stand count after 10 days followed by Vitavax, Plantvax, and Panogen 15, respectively. As seed treatments, Demosan protected cotton seedlings for about 10 days; Vitavax for about 3 weeks; and Plantvax about 2 weeks under laboratory conditions. Thus, these techniques were found to be suitable for comparing and evaluating the systemic activity of several systemic fungicides.

Using the same experimental design, but using either nonsterile or sterile soil in the greenhouse instead of vermiculite Demosan at 9 oz/100 lb gave better and longer protection than when incorporated in soil at .32 gm/pot. Vitavax and Plantvax gave better protection when they were used in soil treatments at .15 gm/pot than when they were used as seed treatments at 4 oz/100 lb. Similar results were obtained by Borum and Sinclair (11) who found that Vitavax gave greater disease control as soil treatments than as any seed treatments for a period up to 25 days. Sinclair, Sloane, and Melville (124) showed in recent studies in Louisiana that Vitavax, either as a seed or soil treatment, was effective in controlling cotton seedling diseases under field conditions. Cotton seedlings are susceptible to many soil-borne pathogens, but R. solani is one of the chief causes of damping-off in Louisiana soils (116). It was suggested from the results presented in this thesis that at least a portion of protection provided by Vitavax under field conditions was due to the control of R. solani.

Edgington et al. (41) demonstrated Vitavax and its sulfone analog Plantvax to be highly selective for most Basidiomycetes. It was shown that these compounds were especially effective against the organisms causing rusts and smuts (42, 63) as well as against species of Rhizoctonia in vitro (11, 111, 122, 124, 139).

From previous results it was observed that Demosan appeared to stimulate germination. Seed treated with Demosan tended to germinate and emerge earlier than nontreated seed. This fungicide showed systemic activity in cotton seedlings against R. solani for about

2 weeks when MD cottonseed was treated at a rate of 9 oz/100 lb under greenhouse conditions, and 10 days under laboratory conditions, but it gave protection for about 30 days when the compound was incorporated in the vermiculite in which plants were grown. Certain environmental factors such as temperature and moisture appeared to have an effect on the activity of Demosan. Research results from the E. I. duPont Company showed that Demosan is not active under low temperatures.

Vitavax tended to delay germination about 2 to 3 days when used as a seed or soil treatment. This fungicide showed systemic activity for over 3 weeks when used as seed treatment. When incorporated in either sterile or nonsterile soil at a rate of .15 gm/pot, seedlings were protected for over 4 weeks.

Plantvax tended to delay germination about 3 to 4 days when used as seed or soil treatments and was observed to cause stunting. It was suggested that this fungicide at the rates used (4 oz/100 lb or .15 gm/pot) may cause death of weak seed. Phytotoxicity was more evident when AD cottonseed was used as compared to MD seed. It was difficult to evaluate disease control because of phytotoxicity. Plantvax gave protection for about 3 weeks. It would not be recommended for use on cotton because of these results.

Results obtained from the above studies indicated that Demosan was both systemic in cotton seedlings and an effective chemotherapeutic agent against R. solani. It was decided to make further studies to meet the criteria for a systemic fungicide. Four series of experiments were conducted to: (1) determine what histological effects, if any, Demosan had on cotton seedlings; (2) bioassay

Demosan-treated, cotton seedlings to determine if fungicidal activity could be detected in treated seedlings; (3) conduct in vitro studies and determine if Demosan had direct action on the fungus; and (4) test for any effects on reducing sugars in cotton seedlings treated with Demosan.

For the histological studies, samples of hypocotyl tissue were taken at the end of each flask experiment from different treatments. Sections of seedling hypocotyls which came in direct contact with the test fungus, showed that Demosan apparently did not alter the physical structure of cotton hypocotyls regardless of concentrations used. These studies showed that the protection to the hypocotyl against R. solani increased when the concentration of Demosan increased. Results of the flask technique and the histological studies showed that Demosan apparently moved through the all of the hypocotyl and protected them from invasion by the test fungus. Transverse sections of infected hypocotyl tissue from nontreated inoculated checks showed invading hyphae growing through epidermal cells into cortical cells, phloem cells, and through the vascular cylinder into pith cells. The host tissue became completely disintegrated to vascular cells except for xylem tissue, which apparently was not attacked. This is in agreement with the results of Khadga et al. (81). Transverse sections of infected hypocotyl tissues treated with Demosan at 300 and 600 ppm showed hyphae concentrated in pockets formed in the epidermis and first layers of cortical cells. More invasion by hyphae was noted in plants treated with Demosan at 300 than at 600 ppm. The fungus did not invade the epidermal cells of seedlings treated with 900 and

1200 ppm Demosan. Mycelium grew only on the hypocotyl surface. Observation by Borum (10) showed that in Vitavax-treated cotton seedlings that at 10 ppm R. solani grew on the surface of the seedling but did not enter.

In the bioassay experiment of Demosan-treated cotton seedlings, the results showed a significant decrease in radial growth of R. solani when plated on agar containing extracts from cotton seedlings treated with increased concentrations of the fungicide. These results indicated that Demosan was probably absorbed by cotton seedlings and remained in extracts of their tissue. It is still questionable whether Demosan was unchanged in the cotton seedling. The in vitro studies showed that Demosan had a direct action on the fungus.

The results from experiments to determine if Demosan had any effect on the quantity of reducing sugars in cotton seedlings showed that Demosan did reduce the amount of reducing sugars significantly after 15 days from sowing, but not significantly after 7 days. Demosan protected cotton seedlings for about 10 days in the laboratory. These results further showed that the balance between resistance and susceptibility to disease through an altered metabolism may be delicate. the highest amount of reducing sugar was in the nontreated-noninoculated checks, and nontreated-inoculated checks. The highest number of diseased seedlings was in the nontreated-inoculated checks, and this verified that R. solani might be considered a sugar-loving organism. This is in agreement with Guinn and Hunter (54) and Guinn and Stewart (55) who found that chilling the roots of cotton seedlings caused a rapid increase in sugar content of stems, reducing sugars

doubled and nonreducing sugars increased about seven-fold in 2 days. They found that homogenates from chilled stems supported almost twice as much growth as seedling-disease fungus, R. solani, as did the homogenate from unchilled plants, by other words they found that tissue high in sugar was attacked by the fungus more than tissue low in sugar. These results were in agreement with Dimond (25), and Horsfall and Dimond (73) who stated that the sugar content of tissue is a useful index, whether or not it determines resistance or susceptibility as such. This relationship may offer a useful approach to chemotherapy by showing if the fungicide acts directly upon the pathogen or acts indirectly by altering the metabolism of the host itself by causing it to become more resistant to disease.

It is concluded that Demosan or a compound related to it, acts as a systemic fungicide for the protection of cotton seedling hypocotyls against infection by R. solani. The conclusions are based on the criteria set up by Dimond et al. (28) for systemic fungicidal action. These are:

1. The compound must be absorbed and enter into the host plant;

2. The compound must be translocated from the point of entry to at least as far as the locus of infection; and

3. The compound must act directly upon the pathogen by virtue of its fungitoxic properties.

A fourth criterion might be that the compound remains unchanged in the host.

Demosan met the three criteria set up by Dimond et al. (28) in the studies reported in this dissertation. Results from various laboratory

and greenhouse techniques showed that Demosan or a compound related to it, was absorbed by the seedling roots and translocated to the hypocotyl where it protected the host tissue for a time from invasion by R. solani. Of the techniques used, the flask technique gave the best evidence of this phenomenon because it widely separated the fungicide solution from the fungus and locus of infection.

Further evidence of translocation was presented by Fielding and Rhodes (47), who showed that radioactive Demosan was absorbed by roots of bean seedlings and translocated to the hypocotyls and cotyledons. These workers showed systemic activity in cucumber seedlings. They did not work with cotton seedlings.

Histological studies showed that as the concentration of Demosan was increased, the greater was the protection. This indicated that Demosan or a compound related to it, was translocated to the site of infection and that, presumably, concentration of the fungicide had an effect on uptake and/or absorption.

The results from these two sets of experiments plus those from the bioassay studies meet the first and second criteria of Dimond et al. (28).

Their third criterion is met by results from in vitro studies and the effects of Demosan, or a compound related to it, on reducing sugars. Demosan incorporated into agar significantly inhibited the growth of the test fungus. Since reducing sugars were not affected significantly after 7 days in Demosan-treated seedlings, it might be suggested that the fungicide was acting directly on the fungus at this time rather than effecting the resistance or susceptibility of the host tissue.

The question of whether Demosan was modified in the host tissue or not remains unanswered. We cannot conclude from these data that Demosan did not change into another fungicidal material either while in solution or in the host tissue, but it seems doubtful.

Finally, this study on Demosan showed that it has certain advantages and disadvantages. The apparent advantages are that it:

(1) Stimulated germination of cottonseed;

(2) According to visual observation, stimulated seedling growth and vigor;

(3) Caused very little phytotoxicity except at high concentrations;

(4) Was a systemic fungicide;

(5) Acted directly against R. solani in vitro;

(6) Could be used as either a seed or soil treatment; and

(7) Did not change the physical structure of cotton hypocotyls regardless of concentrations used.

The apparent disadvantages are that it:

(1) Gave only 10 days' protection in the laboratory, and about 2 weeks in the greenhouse against R. solani;

(2) Required high concentrations for complete systemic activity in cotton seedlings against R. solani;

(3) Gave better control in sterile soil than in nonsterile soil and this may make it less effective against R. solani under field conditions; and

(4) Caused stunting when used at high concentrations.

SUMMARY

1. Techniques were evaluated for their effectiveness to screen systemic fungicides for control of R. solani on cotton seedling hypocotyls.
2. A glass dish and flask techniques using vermiculite were developed for use in the laboratory, and a clay pot technique using soil was developed for use in the greenhouse to study the systemic chemotherapeutic activity of three fungicides against R. solani on cotton seedlings.
3. A greenhouse flat technique proved not to be satisfactory.
4. Using the flask technique Demosan gave complete control at 900, and 1200 ppm for 10 days.
5. Demosan showed systemic activity in cotton seedlings against R. solani for about 2 weeks when MD cottonseed was treated at a rate of 9 oz/100 lb under greenhouse conditions, and 10 days under laboratory conditions.
6. Demosan gave protection for about 30 days when the compound at 600 ppm was incorporated in the vermiculite using the glass dish technique.
7. Demosan gave the highest percentage of healthy seedlings when added to the soil at .64 gm/pot at planting time.
8. Phytotoxicity occurred on seedlings in soil treated with .96 gm/pot Demosan and this reduced both percentage of germination and percentage of healthy seedlings.

9. Demosan appeared to stimulate germination. Seed treated with Demosan tended to germinate and emerge earlier than nontreated seed.

10. Demosan incorporated in sterile soil gave better protection than in nonsterile soil.

11. Three experiments were conducted using the glass dish technique in the laboratory and the clay pot technique in the greenhouse to evaluate Vitavax and Plantvax for their systemic activity in cotton seedlings against R. solani and compare it with Demosan.

    Laboratory experiments showed that Demosan, Vitavax, and Plantvax as seed treatments protected cotton seedlings for about 10 days for Demosan, about 3 weeks for Vitavax, and 2 weeks for Plantvax.

    Greenhouse experiments showed that Demosan gave protection for 2 weeks when MD cottonseed was treated at a rate of 9 oz/100 lb. Vitavax gave protection for over 3 weeks when used as seed treatment, and for over 4 weeks when used as soil treatment. Plantvax gave protection for 2 weeks as seed treatment and for about 3 weeks as soil treatment. Vitavax and Plantvax gave better protection when used as soil treatments at .15 gm/pot than when used as seed treatments at 4 oz/100 lb.

12. Vitavax tended to delay germination about 2 to 3 days when used as seed or soil treatment, and Plantvax tended to delay germination about 3 to 4 days when used as seed or soil treatment.

13. Demosan on MD seed gave both a higher percentage of germination and healthy seedlings when sowed in either noninfested or sterile soil than Vitavax, Plantvax and Panogen 15 on AD seed sowed in infested soil and nonsterile soil.

14. Results from this work coupled with field evaluations gave indication that Demosan, Vitavax, and Plantvax may be developed and used commercially for control of cotton soreshin.
15. Histological studies on plants treated with Demosan showed that the protection to the hypocotyl against R. solani increased when the concentrations increased.
16. In the bioassay experiment of Demosan-treated cotton seedlings, there was decrease in radial growth of R. solani when the extract added to the media came from seedlings treated with high concentrations of the fungicide. These results showed that Demosan was absorbed by cotton seedlings and the tissue extract from treated seedlings had direct effect against R. solani.
17. In vitro studies with Demosan incorporated in agar also showed that Demosan acted directly against R. solani.
18. Demosan reduced the amount of reducing-sugars significantly after 15 days from sowing and nonsignificantly after 7 days from sowing. There was a decrease in the amount of reducing sugars in cotton seedlings with an increase in fungicide concentration.

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VITA

Ismail Elsayed Mohamed Darrag was born on November 28, 1934, in Minya el Qamh, Egypt, U. A. R. He received his secondary education in the public schools of Cairo and Giza graduating from Saideiah Secondary School in 1954. In the same year he enrolled in the Faculty of Agriculture, Ain Shams University from which he obtained his B.Sc. in Plant Pathology in June, 1958. In March, 1959 he was employed by the Egyptian Agricultural Organization, Cairo, to work in Bahtim Experiment Station on cotton diseases. In 1960, he entered the Faculty of Agriculture, Ain Shams University, Cairo, U. A.R., and received a M. Sc. degree in Plant Pathology in May, 1963. In 1964, he was awarded a study leave from the Egyptian Agricultural Organization to study towards a Ph.D. degree in cotton diseases. In September, 1964, he entered the Graduate School of Louisiana State University, where he is now a candidate for the degree of Doctor of Philosophy in the Department of Botany and Plant Pathology.

He married Dorreiah E. Salem in 1964, and has one child (Ahmed).

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1. Ashour, W. A., A. R. Sirry, H. A. Mohamed, and I. E. Darrag. 1964. Studies on the Fusarium wilt of cotton in the U.A.R. II. Effect of potassium and phosphatic fertilizers. Bahtim Agric. Exp. Station Tech. Bull. No. 68.

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EXAMINATION AND THESIS REPORT

Candidate: Ismail Elsayed Mohamed Darrag

Major Field: Plant Pathology

Title of Thesis: Chemotherapeutic Activity of 1,4-Dichloro-2,4-Dimethoxybenzene (Demosan) and Other Compounds Against Rhizoctonia solani in Cotton Seedlings

Approved:

James B. Sinclair
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

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Date of Examination:

December 18, 1967